IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of
MASAHIRO IMOTO et. al.
Serial No. 10/009,477
Filed: December 11, 2001
For: CYCLIC AMIDINE COMPOUNDS

DECLARATION

I, Yoshihiro Tani, Ph.D., a citizen of Japan residing at 25-9, Tamasecho, Ibaraki-shi, Osaka, 567-0893, Japan, declare as follows.

1.   I graduated from Faculty of Pharmaceutical Sciences, Osaka University of Pharmaceutical Sciences in 1984.

2.   I graduated from Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University in 1986.

3.   I entered Suntory Limited as a researcher at the Suntory Institute for Biomedical Research in 1987, and have been assigned as a senior researcher of pharmaceutical research laboratories since 1994.

4.   I obtained the Ph.D. in 1991 from Kyushu University.

5.   I worked as a researcher at Uppsala University PET center, Sweden from 1991 to 1992.

The following is my opinion regarding the inventions of this application based on my own knowledge applying the technical background.

Neuronal nicotinic receptor agonists have attracted much interest as potential therapeutic agents for the treatment of cognitive impairments associated with Alzheimer's disease, schizophrenia and Parkinson's disease. Clinical studies have revealed that (-)-nicotine is effective to ameliorate memory and attention deficits in Alzheimer's disease patients (Newhouse et al 1986; Sahakian et al 1989; Jones et al 1992). In animals, (-)-nicotine has been reported to show beneficial effects on memory in aged monkeys and to reverse spatial memory deficits in rat with an experimental

lesion of the medial septal nucleus (Levin 1992; Decker et al 1995). In addition, the centrally acting nicotinic receptor channel blocker, mecamylamine, produces significant cognitive impairment that mimics certain aspects of Alzheimer's disease in young and elderly volunteers (Newhouse et al 1994). Postmortem studies of Alzheimer's disease brain tissue demonstrated marked reductions of nicotinic receptors in both neocortex and hippocampus, consistent with the Alzheimer's disease pathology of neuronal degeneration (Araujo et al 1988). These findings point to the functional importance of nicotinic acetylcholine systems in cognitive functions (for recent reviews, see Rezvani and Levin 2001; Newhouse et al 2001).

Regarding the several types of neuronal nicotinic receptor ligands recently discovered, extensive pharmacological and behavioral studies have been carried out on (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418) (Garvey et al 1994), selective agonist at $\alpha 4 \beta 2$ subunits more than $\alpha 3$ and $\alpha 7$ subunit of neuronal nicotinic receptor (Americ et al 1994). ABT-418 showed potent cognition-enhancing properties in improving retention of avoidance learning in normal mice and attenuated lesion-induced deficits in a spatial memory in animal model of Alzheimer's disease (Decker et al 1994a; Decker et al 1994b). ABT-418 was the first novel selective nicotinic agonist tested in human patients. In placebo-controlled design study, ABT-418 showed significant dose-related improvement in learning and memory in early to moderate Alzheimer's disease patients (Potter et al 1999). However, ABT-418 is no longer in development by Abbott due to lack of oral bioavailability and the separation between the therapeutic effect and potential cardiovascular side effects were too small to be acceptable.

Clinical studies indicate that (-)-nicotine may be beneficial for the treatment of impairment in attention and rapid information processing associated with Alzheimer's disease, and imply that not only the cholinergic system but also monoaminergic systems are possible mechanisms by which (-)-nicotine treatment improves cognitive performance. Among the monoaminergic systems, it has been suggested that noradrenergic effects of stimulants as important therapeutic mechanisms on enhancing capacities such as attention and working memory.

Therefore, after performing the binding assays at two types of nicotinic receptors and agonist activities at human $\alpha 4 \beta 2$ subunits of nicotinic receptors using Xenopus oocytes, we evaluated the effects of the compounds of the present invention on norepinephrine (NE) turnover in the mouse whole brain as the first *in vivo* assay. It has been reported that brain NE turnover is enhanced by systemic administration of (-)-nicotine in various brain regions and its effect is blocked by the centrally acting nicotinic receptor channel

blocker, mecamylamine (Morgan and Pfeil 1979; Kubo et al 1989). The effects of nicotinic receptor agonists such as (-)-nicotine and ABT-418 were also evaluated as reference compounds.

Materials and methods

Male ddY mice (6 weeks of age, Nihon SLC, Shizuoka, Japan) were used. They were housed in climate-controlled room (room temperature $23 \pm 1^{\circ}C$ and humidity 55 ± 5 %) and allowed free access to food and water. Mice were killed by decapitation, after 30 min the compound or drug was administered subcutaneously (s.c.). The mouse whole brain was homogenated in 2.0 ml of 0.1 M perchloric acid containing 0.1 % $Na_2S_2O_5$ and 0.1 % EDTA2Na, followed by the addition of 3,4-dihydrobenzylamine (DHBA) 50 ng as the internal standard. After centrifugation at 1000 g for 20 min, the supernatant was stored at -80 $^{\circ}C$ until assay. The concentrations of NE and 3-methoxy-4-hydroxy-phenylglycol (MHPG), the NE metabolite, were determined by use of a liquid chromatography (LC) system with electrochemical detection. The LC system consisted of a PM-60 pump (BAS) set at 1.2 ml/min, connected to a reverse-phase column (Cosmosil 5C18, 250 mm x 4.6 mm i.d., 5μ m) maintained at 35$^{\circ}C$ with a column heater (LC-22A, BAS). NE and MHPG were detected with an electrochemical detector (LC-4B, BAS) set at a potential 750 mV versus the Ag/AgCl reference electrode. The mobile phase was 0.1 M sodium acetate/citric acid buffer, pH 4.80 containing 8 % methanol and 4.6 mM sodium 1-octanesulfonate. The results were statistically analyzed using the Dunnett's two-tailed multiple comparison test. A probability level of $p < 0.05$ was considered significant.

Results and Discussion

The dose-response studies of the compound No.2 (0.04 – 5.0 mg/kg s.c.) of the present invention, compound No.3 (0.2 – 5.0 mg/kg s.c.) of the present invention, (-)-nicotine (0.04 – 5.0 mg/kg s.c.) and ABT-418 (0.2 – 5.0 mg/kg s.c.) on NE turnover in the whole brain of mice were performed (Table 1). (-)-Nicotine increased both MHPG content and MHPG/NE ratio in a dose-dependent manner, and showed significant increases at doses of 1.0 and 5.0 mg/kg. The selective agonist at $\alpha 4\beta 2$ subunits, ABT-418 showed enhancement of both MHPG content and MHPG/NE ratio in a dose-dependent manner, but significant increase was observed at the highest dose of 5.0 mg/kg. The compounds of the present invention also increased both MHPG content

3

and MHPG/NE ratio in a dose-dependent manner. The compound No.2-induced changes in NE turnover were similar to those of (-)-nicotine. The compound No.3 significantly increased MHPG content at 1.0 and 5.0 mg/kg, and significant increase of MHPG/NE ratio was observed at 5.0 mg/kg.

Table 1   Effects of (-)-nicotine, ABT-418 and the compounds of the present invention on NE turnover in the mouse whole brain.

Compound	Dose	MHPG	NE	MHPG/NE
	(mg/kg s.c.)	(% of saline group)		
No.2	0.04	118.9±4.6	106.1±4.3	112.2±3.1
	0.2	126.4±8.0	107.9±3.5	116.7±6.1
	1.0	151.1±6.3 *	109.1±3.3	138.1±3.8 *
	5.0	213.0±12.5 *	101.2±3.1	211.5±14.6 *
No.3	0.2	105.5±6.4	102.5±3.1	102.7±6.1
	1.0	130.8±4.6 *	105.6±2.0	123.4±4.0
	5.0	194.9±13.7 *	99.7±2.0	193.7±11.2 *
(-)-nicotine	0.04	106.0±3.7	99.4±3.1	107.1±5.4
	0.2	107.0±6.6	101.8±4.2	105.0±5.9
	1.0	143.5±0.4 *	100.2±4.5	142.3±2.2 *
	5.0	226.9±19.9 *	93.1±3.1	240.5±14.1 *
ABT-418	0.2	104.1±2.6	101.0±3.3	103.3±2.3
	1.0	109.8±4.7	95.9±2.0	114.3±3.7
	5.0	160.1±5.7 *	104.0±2.8	154.6±7.0 *

Animals were killed 30 min after the compound or drug administration. Values in the Table are expressed as percent change from control levels 30 min after saline treatment. * $p < 0.05$; significantly different from saline group (Dunnett's two-tailed test, mean ± SEM, n=8).

Among novel nicotinic receptor agonists, ABT-418 showed significant improvement both in experimentally induced animal models (Decker et al 1994b) and in early to moderate Alzheimer's disease patients (Potter et al 1999). ABT-418 was a selective agonist at α4β2 subunits of nicotinic receptor, since ABT-418 had high affinity for [^{3}H]cytisine binding (Ki = 3 nM) but was inactive in 37 other receptor, neurotransmitter-uptake, enzyme, transduction system binding assays, and ABT-418 was equipotent to (-)-nicotine in stimulating [^{86}Rb$^+$] efflux from mouse thalamus that was thought to reflect the activation of α4β2 subunits of nicotinic receptor (Americ et al 1994). We also confirmed such abilities of ABT-418 using receptor binding assays and

Xenopus oocytes expressing α4β2 subunits of nicotinic receptor.

Regarding the mechanisms by which (-)-nicotine enhanced brain NE turnover, previous our studies have indicated that (-)-nicotine enhances brain NE turnover may be attributed to activation of α4β2 subunits but not α7 nicotinic receptors (Tani et al 2002). Because, (-)-nicotine-induced increase in NE turnover was blocked dose-dependently by pretreatment with dihydro-β-erythroidine (DHβE), a competitive nicotinic receptor antagonist that reported to be more sensitive to α4β2 subunits, but a selective nACh-R antagonist for α7 subunit, methyllycaconitine (MLA) did not affect (-)-nicotine-induced increase in NE turnover. The neuronal nicotinic receptors are thought to be composed of α and β subunit and the most abundant nicotinic receptor in the central nervous system consists of α4 and β2 subunits (Flores et al 1992), while in recombinant expression system α7 subunit can form functional homooligomeric receptor. What subunits of nicotinic receptors might mediate cognition-enhancing properties is as yet unclear, but α4β2 subunits of nicotinic receptor appear to have the greatest relevancy to Alzheimer's disease and other cognitive disorders (Newhouse et al 2001).

The compounds No.2 and No.3 of the present invention had high affinity for [^{3}H]cytisine binding. Studies with Xenopus oocytes expressing α4β2 subunits of nicotinic receptor also demonstrated that the compounds No.2 and No.3 had the abilities as agonists for α4β2 subunits of nicotinic receptor. The present study indicated that a singe systemic administration of compound No.2 and No.3 enhanced brain NE turnover in a dose dependent manner and the relative potencies for enhancement of brain NE turnover were (-)-nicotine = compound No.2 and No.3 > ABT-418. Therefore, these findings suggest that the compounds No.2 and No.3 of the present invention may exhibit potent cognition-enhancing properties in animal model of Alzheimer's disease.

References

Araujo, D.M., Lapchak, P.A., Robitaille, Y., Gauthier, S., Quirion, R., 1988. Differential alternation of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. J. Neurochem. 50, 1914-1923.

Americ, G.W., Sullivan, J.P., Briggs, C.A., Donnelly-Roberts, D., Anderson, D.J., Raszkiewiez, J.L., Huges, M.L., Cadman, E.D., Adams, P., Garvey, D.S., Wasicak, J.T., Williams, M., 1994. (S)-3-Methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: I. In vitro characterization. J. Pharm. Exp. Ther. 270, 310-318.**
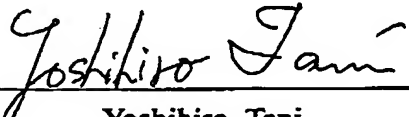
Decker, M.W., Brioni, J.D., Sullivan, J.P., Buckley, M.J., Radek, R.J., Raszkiewicz, J.L., Kang, L.H., Kim, D.J.B., Giardina, W.J., Wasicak, J.T., Garvey, D.S., Williams, M., Americ, S.P., 1994a. (S)-3-Methyl-5-(1-methyl-2-pyrrolidinyl)-isoxazole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: II. In vivo characterization. J. Pharm. Exp. Ther. 270, 319-328. **

Decker, M.W., Curzon, P., Brioni, J.D., Americ, S.P., 1994b. Effects of ABT-418, a novel cholinergic channel ligand, on place learning in septal-lesioned rats. Eur. J. Pharmacol. 261, 217-222. **

Decker, M.W., Brioni, J.D., Bannon, A.W., Americ, S.P., 1995. Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. Life Sci. 56, 545-570.

Flores, C.M., Rogers, S.W., Pabreza, Z.A., Wolfe, B.B., Kellar, K.J., 1992. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is upregulated by chronic nicotine treatment. Mol. Pharmacol. 41, 31-37.

Gravey, D.S., Wasicak, J., Decker, M.W., Brioni, J.D., Sullivan, J.P., Carrera, G.M., Holladay, M.H., Americ, S.P., Williams, M., 1994. Novel isoxazoles which interact with brain cholinergic channel receptors that have intrinsic cognitive enhancing and anxiolytic activities. J. Med. Chem. 37, 1055-1059.

Jones, G.M.M., Sahakian, B.J., Levy, R., Warburton, D.M., Gray, J.A., 1992. Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology 108, 485-494.

Kubo, T., Amano, H., Kurahashi, K., Misu, Y., 1989. Nicotine-induced regional changes in brain noradrenaline and dopamine turnover in rats. J. Pharmacobiodyn. 12, 107-112.

Levin, E.D., 1992. Nicotinic systems and cognitive function. Psychopharmacology 108, 417-431.

Morgan, W.W., Pfeil, K.A., 1979. Mecamylamine blockade of nicotine enhanced noradrenaline turnover in rat brain. Life Sci. 24, 417-420.

Newhouse, P.A., Sunderland, T., Thompson, K., Tariot, P.N., Weingartner, H., Mueller, E.R., Cohen, R.M., Murphy, D.L., 1986. Intravenous nicotine in patient with Alzheimer's disease. Am. J. Psychiatry 143 1494-1495.

Newhouse, P.A., Potter, A., Corwin, J., Lenox, R., 1994. Age-related effects of the nicotine antagonist mecamylamine on cognition and behavior. Neuropsychopharmacology 10, 93-107.

Newhouse, P.A., Potter, A., Kelton, M., Corwin, J., 2001. Nicotinic treatment of Alzheimer's disease. Biol. Psychiatry 49, 268-278.

Potter, A., Corwin, J., Lang, J., 1999. Acute effects of the selective cholinergic channel activator (nicotinic agonist) ABT-418 in Alzheimer's disease. Psychopharmacology 136, 50-58.

Rezvani, A.H., Levin, E.D., 2001. Cognitive effects of nicotine. Biol. Psychiatry 49, 25-267.

Sahakian, B.J., Jones, G., Levy, R., Gray, J.A, Warburton, D.M., 1989. The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of Alzheimer's type. Br. J. Psychiatry 154, 797-800.

Tani, Y., Oka, T., Ohno, T., 2002. Pharmacological characterization of nicotinic acetylcholine receptor (nACh-R)-mediated changes in brain norepinephrine (NE) turnover in mice. Jpn. J. Pharmacol. 88, supple. 189P.

**Note: The three references underlined are attached herein.

I, the undersigned petitioner, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 24 day of January, 2003

_____
Yoshihiro Tani

This 24 day of January, 2003

_____
Witnessed by Toshio Tatsuoka, Ph.D.
General Manager

7

(S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl) Isoxazole (ABT-418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: I. In Vitro Characterization

STEPHEN P. ARNERIC, JAMES P. SULLIVAN, CLARK A. BRIGGS, DIANA DONNELLY-ROBERTS, DAVID J. ANDERSON, JOANNA L. RASZKIEWICZ, MARION L. HUGHES, EVELYN D. CADMAN, PAMELA ADAMS, DAVID S. GARVEY, JAMES T. WASICAK and MICHAEL WILLIAMS

Neuroscience Discovery, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois

Accepted for publication March 3, 1994

ABSTRACT

A diversity of nicotinic acetylcholine receptor (nAChR) subtypes has been identified in mammalian brain using recombinant DNA technology. Alterations in the activity of these acetylcholine-gated ion channels have been implicated in a number of central nervous system disorders including Alzheimer's disease (AD). The potential therapeutic usefulness of (−)-nicotine [(S)-3-(1-methyl-2-pyrrolidinyl) pyridine], the prototypic agonist at nAChRs, is severely limited by side effects that are the result of activation of both cholinergic and noncholinergic pathways in the central and peripheral nervous systems. This study sought to determine the in vitro selectivity of (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT 418), a novel analog of (−)-nicotine in which the pyridine ring was replaced with an isoxazole bioisotere, to activate nAChRs. ABT 418 was a potent inhibitor of [^{3}H]-cytisine binding to nAChR in rat brain ($K_i = 3$ nM) but was inactive ($K_i > 10,000$ nM) in 37 other receptor/neurotransmitter-uptake/enzyme/transduction system binding assays, including those for α-bungarotoxin, muscarinic and 5-hydroxytryptamine$_3$ receptors. In PC12 cells, patch-clamp studies indicated that ABT 418 was an agonist with an EC$_{50}$ value of 209 μM, a potency to activate cholinergic channel currents some 4-fold less than that of (−)-nicotine (52 μM). Channel current responses elicited by ABT 418 were prevented by the cholinergic channel blocker, mecamylamine. ABT 418 was also approximately 10-fold less potent (EC$_{50}$ value = 380 nM) than (−)-nicotine (40 nM) in increasing [^{3}H]-dopamine release from rat striatal slices, an effect that was blocked by the nAChR antagonist, dihydro-β-erythroidine (10 μM). In contrast, ABT 418 appeared equipotent with (−)-nicotine in enhancing ^{86}Rb$^+$ flux from mouse thalamic synaptosomes. ABT 418 demonstrated an in vitro pharmacological profile of cholinergic channel activation that was robust at some nAChR, but not others. The reasons for this are unclear. However, a nAChR subtype selectivity may account for the in vitro potency differences of ABT 418 on various neurotransmitter systems, and the substantial separation between the cognitive enhancement/anxiolytic benefits, and the reduced central nervous system side-effect liabilities seen in vivo. ABT 418 represents the first neuronal nAChR ligand that differentiates the toxicities/liabilities and other negative aspects normally associated with (−)-nicotine from the potential pharmacological benefits of selective cholinergic channel activation.

Neuronal nAChRs in the CNS represent an expanding area of potential therapeutic opportunity driven by new findings in the the molecular biology of the system (Changeux et al., 1992; Deneris et al., 1991; Sargent, 1993). The pharmacological properties and physiological function of these newly identified molecular targets remain, to a large extent, unknown primarily due to the lack of potent and selective pharmacological probes. Nonetheless, alterations in the activity of the acetylcholine-gated ion channels have been implicated in a number of CNS disorders including AD (Arneric and Williams, 1994). Preliminary clinical data indicate that acutely administered (−)-nicotine, the prototypic agonist for nAChR, may be beneficial for the treatment of the deficits in attention and information processing associated with AD (Wesnes and Warburton, 1984; Newhouse et al., 1988; Sahakian et al., 1989; Jones et al., 1992). Compounds that selectively interact with subtypes of nAChR to normalize CNS functions governed by this receptor family may, therefore, lead to more effective therapeutic agents (Arneric et al., 1995).

An emerging diversity of alpha and beta nAChR subunits

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor(s); CNS, central nervous system; AD, Alzheimer's disease; 5-HT, 5-hydroxytryptamine (serotonin); α-Bgt, α-bungarotoxin; ABT 418, (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride; A-81754, (R)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride; DHβE, dihydro-β-erythroidine; PE1, polyethylenimine; DMEM, Dulbecco's modified Eagle's medium; ChCA, cholinergic channel activator(s).

have been identified in brain and autonomic ganglia using recombinant DNA technology (Deneris *et al.*, 1991). The nAChR found at the neuromuscular junction is a pentamer comprised of *alpha, beta, gamma, delta* and *epsilon* subunits that demonstrates developmental regulation of subunit composition (Mishina *et al.*, 1986; Changeux, 1990). However, the precise composition of the brain receptors is much less well characterized. Ten gene products *alpha* 2 through *alpha* 8, and *beta* 2 through *beta* 4 have been identified by the cloning of mammalian brain cDNA. Although many of the isolated subunits can form a functional nAChR when reconstituted in pairwise combinations in *Xenopus* oocytes (Sargent, 1993), the stoichiometry of these nAChR *in situ* has yet to be elucidated. At least seven potential neuronal nAChR subtypes have been identified in oocyte preparations (Luetje and Patrick, 1991; Courturier *et al.*, 1990) which have physiological and pharmacological properties that are similar to those native receptors found in various CNS preparations (Mulle *et al.*, 1991; Alkondon and Albuquerque, 1993). The differing pharmacological profile of these functional nAChR suggests that they may represent unique targets for the development of CNS agents. Furthermore, the wide distribution of *alpha* 2, *alpha* 3, *alpha* 4, and *beta* 2 transcripts in the brain suggests that the neuronal nAChR are a neurotransmitter receptor superfamily that may be of global functional importance. The identification of physiological roles for these receptor subtypes presents a challenge and a therapeutic opportunity akin to the identification and development of selective ligands with demonstrated therapeutic utility for the ever expanding 5-HT-receptor superfamily (Zifa and Fillion, 1992).

One approach to identifying subtype selective ligands for nAChR is to evaluate the activity of compounds in adequate models of brain nAChR function—preferably those that contain native receptors found in various CNS preparations. Using receptor binding techniques, two major pharmacological subclasses of nAChR can be clearly delineated in mammalian brain (Clarke *et al.*, 1985; Marks *et al.*, 1986), *i.e.*, those that recognize α-Bgt with high affinity (α-BgtnAChR) and those that do not (nAChR). α-BgtnAChR do not display high-affinity binding for ligands like (−)-nicotine (Wonnacott, 1986), whereas nAChR do (Whiting and Lindstrom, 1986, 1988). The functional pharmacological extension of these receptor binding studies have been accomplished through the identification of *in vitro* preparations using electrophysiological (Pereira *et al.*, 1993; Alkondon and Albuquerque, 1993; Schrattenholz *et al.*, 1993; Papke, 1993), and biochemical (Whiting and Lindstrom, 1986, 1988; Schoepfer *et al.*, 1990; Listerud *et al.*, 1991; Flores *et al.*, 1992; Grady *et al.*, 1992; Marks *et al.*, 1993) techniques that demonstrate selective responsivity for subtypes of nAChR. For example, more than 90% of nicotinic receptor binding with [³H]cytisine occurs at nAChR that contain the *alpha* 4/*beta* 2 subunits (Flores *et al.*, 1992). Correspondingly, this receptor subtype appears to mediate a flux of monovalent ions as measured by efflux of ⁸⁶Rb⁺ from mouse thalamic synaptosomes (Marks *et al.*, 1993). On the other hand, stimulation of [³H]-dopamine release in the striatum appears to be mediated by nAChR containing the *alpha* 3 subunit (Grady *et al.*, 1992). PC12 cells also contain the *alpha* 3, but little or no *alpha* 4, subunit isoforms (Rogers *et al.*, 1992), and can be readily evaluated using whole-cell patch-clamp techniques. In contrast, [¹²⁵I]α-Bgt labels nAChR formed by the *alpha* 7 subunit isoform found in brain (Courturier *et al.*, 1990), and the *alpha* 1 isoform

in the neuromuscular junction and in *Torpedo californica* (Changeaux, 1990). Functionally, the *alpha* 7 homo-oligomer expressed in oocytes has a calcium permeability greater than neuromuscular receptors and, in some instances, greater than N-methyl-D-aspartate channels (Seguela *et al.*, 1993).

In this paper, the *in vitro* characterization of the novel nicotinic receptor ligand ABT 418 is described using tissue preparations that are recognized for their ability to express differentially subunit isoforms of nAChR and serve as bioassays to evaluate potentially selective activators of cholinergic channels. ABT 418 is a novel bioisostere of (−)-nicotine (Fig. 1; Garvey *et al.*, 1994) which, while sharing many of the positive CNS attributes of (−)-nicotine, has a reduced propensity to elicit the side effects that limit the usefulness of (−)-nicotine for the treatment of AD. In the accompanying paper (Decker *et al.*, 1994), the behavioral, cerebral circulatory, electroencephalographic and pharmacokinetic properties of this cholinergic ligand are described.

Materials and Methods

All animal studies were conducted in accord with American Association for the Accreditation of Laboratory Animal Care (AAALAC) procedures as approved by the Institutional Animal Care and Use Committee at Abbott Laboratories.

Compounds. ABT 418 and the *R*-isomer of ABT 418, A-81754 [(*R*)-3-methyl-5-(1-methyl-2-pyrrolidinyl), *isoxazole hydrochloride*] were synthesized as described by Garvey et al. (1994). (−)-Nicotine di-(+)-tartrate salt, (+)-nicotine di-*p*-toluoyltartrate salt, mecamylamine hydrochloride, atropine and urethane were obtained from Sigma (St. Louis, MO). DHβE, MDL 72222 and diazepam were obtained from Research Biochemicals Int (Natick, MA). α-Bgt was obtained from Biotoxin Inc. (St. Cloud, FL). All radioligands were obtained from Du Pont-NEN (Boston, MA).

Stock solutions of ABT 418, A-81754, atropine and (−)-nicotine were prepared in distilled water. DHβE and MDL 72222 were prepared as 10 mM stock solutions in 100% dimethyl sulfoxide.

Receptor Binding

Membranes were prepared from whole rat brains (male Sprague-Dawley rats: 250–400 g; Sasco, Madison, WI) by the method of Enna and Snyder (1977). Brains were rapidly removed after decapitation homogenized in 15 vol of 0.32 M sucrose, and centrifuged at 1000 × g for 10 min at 4°C. The supernatants were removed and centrifuged at 20,000 × g for 20 min at 4°C. The resultant P₂ pellets were homogenized with a Polytron (10 sec, setting of 6) in ice-cold water and spun at 8,000 × g for 20 min. The supernatant and loose buffy coat were carefully removed and centrifuged at 40,000 × g for 20 min. The membrane pellet was washed with ice-cold H₂O and recentrifuged at 40,000 × g before storage at −80°C.

ABT 418
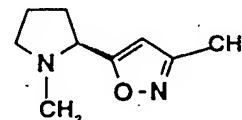
(*S*)-3-Methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole

————-(-)--Nicotine ————
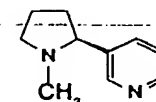
(*S*)-3-(1-methyl-2-pyrrolidinyl) pyridine

Fig. 1. Structures of ABT 418 and (−)-nicotine.

[³H]Cytisine binding. [³H]Cytisine binding was performed using a modification of the method of Pabreza *et al.* (1991). Membrane enriched fractions were slowly thawed at 4°C and washed and resuspended in 30 vol of BSS-Tris buffer (BSS; 120 mM NaCl, 5 mM·KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 50 mM Tris-Cl, pH 7.4, 4°C). Aliquots of protein (100–200 µg), 1.25 nM [³H]cytisine (30 Ci/mmol) and compounds to the final concentrations indicated were incubated in a final volume of 500 µl for 75 min at 4°C in duplicate. Nonspecific binding was determined in the presence of 10 µM (−)-nicotine. Bound radioactivity was separated under vacuum onto #32 glass fiber filters (Schleicher and Scheull, Keene, NH) using a Skatron filtration apparatus (Skatron, Sterling, VA). Filters were prerinsed with 0.5% PEI before sample filtration to reduce non-specific binding and were then rapidly rinsed with 4.5 ml of ice-cold BSS. Filters were counted in 3 ml of Ecolume (ICN, Costa Mesa, CA) at an efficiency of approximately 45% by conventional liquid scintillation counter (model LS5000 TD, Beckman Instruments Inc., Fullerton, CA).

[¹²⁵I]α-Bgt binding. [¹²⁵I]-α-Bgt binding was determined in membranes prepared from whole rat brain and from *Torpedo Californica* electroplax.

α-Bgt binding to rat brain membranes was determined using a modification of the method of Marks *et al.* (1986). Rat brain membranes were resuspended in 15 vol of assay buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM HEPES, pH 7.5). Aliquots containing 200 µg of tissue were added to a reaction mixture containing 1.9 nM [¹²⁵I]α-Bgt (106 Ci/mmol) and the indicated concentrations of ABT 418 or reference agents in triplicate. Nonspecific binding was determined in the presence of 1 µM unlabeled α-Bgt. Binding was conducted at 37°C for 3 hr. Bound radioactivity was isolated by rapid vacuum filtration onto #32 glass fiber filters (Schleicher and Scheull) using a Skatron filtration apparatus. Filters were prerinsed with 0.05% PEI and were then rapidly rinsed with 4.5 ml of ice-cold assay buffer. Radioactivity was measured in a gamma counter (model 5000, Beckman, Fullerton, CA).

A solid phase binding assay was used to measure the binding of [¹²⁵I]α-Bgt (106 Ci/mmol) to the α-BgtnAChR isolate from *Torpedo Californica* electroplax. The wells of a 96-well microtiter plate (Immulon Removawells Strips, Dynatech, Chantilly, VA) were coated with 0.5 µg of *Torpedo* membranes (ABS Inc., Wilmington, DE) in 50 mM $NaHCO_3$ buffer, pH 9.6, for 12 hr at 4°C. Wells were then washed twice with phosphate-buffered saline and quenched for 1 hr with 5% bovine serum albumin. [¹²⁵I] α-Bgt (~1.9 nM/100 µl 10 mM phosphate buffer, pH 7.4/0.2% bovine serum albumin) was then added to the wells for 1 hr. For competition experiments, increasing concentrations of competitor (50 µl) were added to wells in triplicate followed immediately by 50 µl of [¹²⁵I] α-Bgt and incubated for 1 hr. Nonspecific binding was determined in the presence of 1 µM α-Bgt. After incubation, wells were washed 5 times with phosphate-buffered saline. Individual wells were placed in vials and radioactivity measured in a gamma counter (model 5000, Beckman).

[³H]Oxotremorine-M binding assay. [³H]Oxotremorine-M (87 Ci/mmol) binding to the muscarinic cholinergic receptor was performed in 20 mM Na_2PO_4 buffer, pH 7.4 at 25°C for 45 min using a modification of the method of Birdsall *et al.* (1978). The assay mixture contained 100 µg of rat brain membranes per tube, 2 nM [³H]oxotremorine-M and the indicated concentrations of compounds in triplicate. Nonspecific binding was determined in the presence of 10 µM atropine. Radioactivity was isolated and radioactivity determined as described above.

[³H]Diazepam binding. [³H]Diazepam binding to the central benzodiazepine receptor present in rat brain cerebellar membranes was performed using a modification of the method of Falch *et al.* (1985). Fresh cerebella from adult Sprague-Dawley rats (Sasco, Madison, WI) were homogenized in 10 ml of ice-cold 100 mM Tris-citrate (pH 7.1) using a Polytron (setting 6 for 5 sec). The resultant homogenate was centrifuged at 20,000 × g for 10 min at 4°C and the pellet was washed five times by rehomogenization in ice-cold buffer and recentrifugation.

The final suspension was diluted to 0.5 mg/ml in 100 mM Tris-citrate (pH 7.1) with 150 mM NaCl.

The reaction mixture containing 0.8 nM [³H]diazepam (83.5 Ci/mmol), 100 µg of protein and various concentrations of compound in triplicate was incubated for 30 min at 30°C. Non-specific binding was determined in the presence of 3 µM diazepam. After incubation, the samples were collected on GF/C glass fiber filters (Whatman, Clifton, NJ) using a Skatron filtration apparatus and washed with ice-cold 150 mM Tris-citrate buffer. Filters were placed in 3 ml of Ecolume and radioactivity determined as described above.

[³H]GR-65630 binding. [³H]GR-65630 (62.5 Ci/mmol) binding to the 5HT₃ receptor was determined by the method of Hoyer and Neijt (1988). Cells of the N1E-115 mouse neuroblastoma clonal cell line (ATCC, Rockville, MD) were grown in DMEM supplemented with glutamine, 10% fetal bovine serum, 50 units penicillin/streptomycin, 1 mM sodium pyruvate, 1% nonessential amino acids and 2 mM L-glutamine (Gibco-BRL, Bethesda, MD). Cells were harvested in the presence of trypsin-EDTA (Gibco-BRL) to dissociate the cells from the flask surface followed by centrifugation of the medium at 600 × g for 6 min. The resultant cell pellets were homogenized using a Polytron (setting 6 for 5 sec) in 20 vol of 20 mM HEPES containing 50 mM NaCl (pH 7.5 at 25°C).

For competition experiments, aliquots of the homogenate (equivalent to 200,000 cells/tube) were incubated in the presence of 0.7 nM [³H] GR-65630 and the indicated concentrations of compound in triplicate for 30 min at 25°C. Nonspecific binding was determined in the presence of 10 µM MDL 72222. Bound radioactivity was isolated by rapid vacuum filtration onto GF/B glass fiber filters (Whatman) using a Skatron filtration apparatus. Filters were prerinsed with 0.05% PEI and were then rapidly rinsed with 5 ml of 20 mM HEPES containing 0.9% NaCl at 25°C. Filters were placed in 3 ml of Ecolume and radioactivity determined as described above.

Additional receptor selectivity binding studies. To assess further the selectivity of ABT 418 as an nAChR ligand, the compound was evaluated in a *PROFILE* receptor binding selectivity screen by NovaScreen (Hanover, MD) using standard receptor binding protocols (table 2). ABT 418 was tested at three concentrations (1, 100 and 10,000 nM) in duplicate in 35 binding assays for a number of neurotransmitter receptors, channel proteins, peptide factors, reuptake sites and second messenger systems.

Data analysis. In competition experiments, the drug concentration producing 50% inhibition (IC_{50}) of radioligand binding and the Hill coefficient (n_H) were determined from plots of ($B_0 - B$)/B_0 *vs.* log (concentration of drug), where B_0 and B are specific binding in the absence and presence of competitor, respectively, using a four-parameter logistics program in RS/1 (Bolt Beranek and Newman Inc. Cambridge, MA). Inhibition constant (K_i) values were determined using the Cheng-Prusoff equation ($K_i = IC_{50}/1 + [L]/K_d$, where [L] = free radioligand concentration).

⁸⁶Rb⁺ Efflux

The ability of ABT 418 and (−)-nicotine to activate ion channels was investigated by measuring efflux of the potassium ion analog ⁸⁶Rb⁺ from mouse thalamus using the methods of Marks *et al.* (1993).

Thalami were dissected from the brains of female C57 BL/61bg mice (60–90 days old; Institute for Behavioral Genetics, Boulder, CO) and homogenized in 10 vol (w/v) of ice-cold 0.32 M sucrose, 5 mM HEPES, pH 7.5 by hand using 16 strokes in a Teflon-glass homogenizer. The homogenate was diluted to 25 vol with ice-cold 0.32 M sucrose and centrifuged for 10 min at 1000 × g and the resulting supernatant recentrifuged for 20 min at 15,000 × g to yield the P_2 pellet. The P_2 fraction was resuspended in 8 vol of ice-cold perfusion buffer (140 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM HEPES, 20 mM D-glucose, pH 7.5).

A P_2 fraction equivalent to two thalami was incubated for 30 min at 21°C in 35 ml of perfusion buffer containing 4 µCi of ⁸⁶Rb⁺ (35 Ci/mmol). At the end of the incubation period, tissue was harvested and separated from the incubation medium by filtration onto 6 mm diam-

eter glass fiber filters (type GC50, Microfiltration Systems, Dublin, CA) under gentle vacuum (−0.2 atm) followed by eight washes at room temperature with perfusion buffer. The filter containing the ^{86}Rb-loaded synaptosomes was placed on a 13-mm glass fiber filter (type GC50, Microfiltration Systems) mounted on the bottom half of a plastic filter holder (Swinney type, 13 mm, Gelman Sciences, Inc., Ann Arbor, MI) modified to reduce the dead volume beneath the filter platform by fusing 14-gauge stainless steel tubing inside the holder with epoxy cement. The filter containing the tissue was subsequently perfused continuously at 21°C. After an initial average wash period of 8 min, fractions were collected every 30 sec by using a Retriver II fraction collector (ISCO, Inc., Lincoln, NE). Exposure to ABT 418 and (−)-nicotine occurred approximately 3 min into a 10-min collection period. In any experiment, five concentrations of each nicotinic receptor ligand were tested and the tissue on each filter stimulated only one time. (−)-Nicotine (10 μM) was included in each experiment as control to normalize values between experiments. Radioactivity was measured using a Packard Auto-Gamma counter (Packard, Naperville, IL) and the magnitude of the ^{86}Rb$^+$ response amplitude calculated by determining the increase in radioactivity above the base line after stimulation of the tissue. The average base line underlying the peak was calculated by averaging the radioactivity present in the tubes immediately before and after the peak. Peak size was determined by subtracting the average base-line value from each fraction in the peak. To correct for differences in total tissue content and base-line release, the response was normalized by dividing by the amount of ^{86}Rb$^+$ present in the tissue at the time of stimulation. Estimates of the EC$_{50}$ values obtained for stimulation of ^{86}Rb$^+$ efflux were calculated using Inplot (Graphpad, San Diego, CA), and relying on the assumption that the highest concentration of (−)-nicotine used in this study elicited a near maximal ion flux (Marks *et al.*, 1993).

Striatal Dopamine Release

nAChR-evoked release of [ring-2,5,6-^{3}H]dopamine (24.4 Ci/mmol) was measured in superfused rat striatal slices. Striata were dissected from two male Sprague-Dawley rats per experiment and sliced 0.35 × 0.25 mm by a McIlwain Tissue Chopper (Brinkman Instrument Co., Westbury, NY). After two washes with Krebs-HEPES buffer (137 mM NaCl, 4.7 mM KCl, 1 mM MgSO$_4$, 2.5 mM CaCl$_2$, 1.25 mM NaH$_2$PO$_4$, 10 mM glucose, 15 mM HEPES-NaOH, pH 7.4, containing 10 μM pargyline and 10 μM ascorbic acid), slices were preincubated for 10 min at 37°C under 95% O$_2$/5% CO$_2$. After replacing the buffer, slices were labeled with 100 nM [^{3}H]dopamine for 25 min in Krebs-HEPES at 37°C. Aliquots of slices were placed in 18 superfusion chambers of a Brandel SP2000 superfusion apparatus (Brandel, Gaithersberg, MD). After 47 min of washout at 0.5-ml/min, slices were exposed to agonist for 4 min. Antagonists, when present were introduced 4 min before and during agonist exposure. Collected 2-min fractions were counted in 5 ml of Ecolume. Tissue was recovered from superfusion chambers, solubilized with 1 ml of Solvable (DuPont-NEN) and counted in 15 ml of Ecolume.

Fractional release of [^{3}H]dopamine was calculated from radioactivity above baseline as a fraction of total radioactivity. Relative potencies were calculated using the release evoked by 100 nM (−)-nicotine as a standard. EC$_{50}$ values were determined by nonlinear least squares regression analysis using InPlot.

Whole-Cell Channel Currents

Rat pheochromocytoma (PC12) cells were obtained from ATCC (Rockville, MD) and maintained in DMEM containing 10% heat-inactivated fetal calf serum and 5% heat-inactivated horse serum (37°C, 95% O$_2$/5% CO$_2$). Nicotinic cholinergic responses were obtained from differentiated (neurite-bearing) cells after 4 to 7 days exposure to mouse nerve growth factor (NGF; Collaborative Biomedical Products, Bedford, MA). For this purpose, the undifferentiated cells were first plated onto poly-L-lysine-coated glass coverslips in plastic Petri dishes. After 20 min, differentiating medium (DMEM containing 100 ng/ml NGF, 5% heat-inactivated fetal calf serum and 2.5% heat-inactivated horse

serum) was added and the cells were refed with this medium every 3 days.

The whole-cell patch-clamp technique was used to record voltage- and ligand-activated currents. A coverslip bearing the cells was transferred from culture dish to the recording chamber (350 μl vol) and superfused (1 ml/min) at room temperature (21–23°C) with an extracellular solution containing 150 mM NaCl, 2.8 mM KCl, 2.0 mM CaCl$_2$, 1.0 mM MgCl$_2$, ≥10 mM dextrose and 10 mM Na-HEPES buffer (7.3 pH, 325 mOsm). The intracellular (recording pipette) solution contained 140 mM KCl, 1.0 mM CaCl$_2$, 2.0 mM MgCl$_2$, 11 mM K-EGTA, and 10 mM K-HEPES buffer (7.3 pH, 315 mOsm). Osmolarities were adjusted using dextrose such that the extracellular solution was 10 mOsm hypertonic relative to the intracellular solution. Voltage-activated currents were monitored throughout the experiment to determine establishment and maintenance of the whole-cell recording configuration.

The cells were kept at a holding potential of −60 mV and cholinergic channel ligands dissolved in bathing solution were applied to the cells through computer-controlled U-tube flow reversal (Fenwick *et al.*, 1982) for a period of 5 sec. Each test compound was applied at least twice at each concentration in every cell, and applications were separated by ≥3 min to allow for recovery from desensitization, washout of the bathing solution and re-equilibration of the U tube. Antagonists were applied to the bathing solution through superfusion for several minutes before application of both antagonist and putative agonist through flow-reversal. Data were acquired and quantified using an Axopatch 1B patch-clamp amplifier, a Tecmar Labmaster 125 KHz A/D system and pClamp software (Axon Instruments, Foster City, CA).

For quantifying dose-response relationships, each PC12 cell was exposed to several different concentrations of (−)-nicotine or ABT 418 and the peak inward currents were measured. To correct for variation in PC12 cell responsiveness each response to various concentrations of (−)-nicotine was normalized to the response to 100 μM (−)-nicotine obtained in the same cell; ABT 418 responses were normalized to 300 μM ABT 418. Furthermore, the standards [100 μM (−)-nicotine or 300 μM ABT 418] were applied at the beginning, end and often middle of every experiment with each cell to evaluate changes in the response of the cell with time. The following equation was fit to the data using a nonlinear curve-fitting program (SigmaPlot, Jandel Scientific, San Rafael, CA):

$$I = I_{max} \times \frac{[A]^{n_H}}{EC_{50}^{n_H} + [A]^{n_H}}$$

where I is the observed current response, I_{max} is the maximal response, [A] is the agonist concentration, EC$_{50}$ is the agonist concentration that produces a half-maximal effect and n_H is the Hill coefficient.

Results

Receptor binding. Both ABT 418 and (−)-nicotine competitively displaced [^{3}H]cytisine in a concentration-dependent manner with respective K_i values of 3.0 ± 0.4 nM ($n = 5$) and 1.0 ± 0.1 nM ($n = 3$; table 1; fig. 2). The concentration-response

TABLE 1

nAChR binding properties of ABT 418 and (−)-nicotine

Values represent mean ± S.E.M. Numbers in parentheses represent number of experiments.

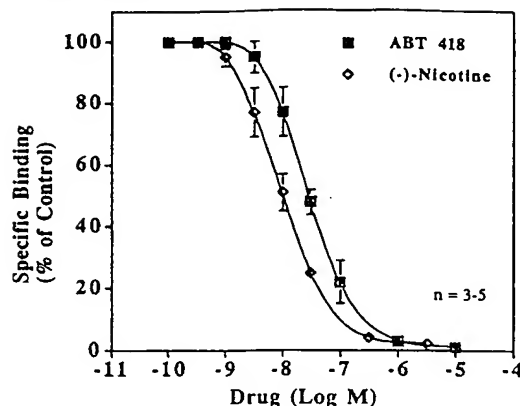|	K_i (nM)		
	[^{3}H]cytisine	[^{125}I]α-Bungarotoxin	
	Rat Brain	Torpedo-Electroplax	Rat Brain
ABT 418	3.0 ± 0.4 (5)	>100,000 (3)	>10,000 (3)
A-81754	44 ± 12 (3)	>10,000 (3)	>10,000 (3)
(−)-Nicotine	1.0 ± 0.1 (3)	>100,000 (3)	4000 ± 800 (3)

Fig. 2. Displacement of [³H]cytisine binding by ABT 418 and (−)-nicotine. Rat brain membranes containing 100 to 200 μg of protein, 1.25 nM [³H] cytisine and the indicated concentrations of drug were incubated in a final volume of 500 μl for 75 min at 4°C. ABT 418 and (−)-nicotine had K_i of 3 ± 0.4 and 1.0 ± 0.1 nM, respectively. Nonspecific binding was determined in the presence of 10 μM (−)-nicotine.

curve for ABT 418 was consistent with a single site competitive model ($n_H = 1.02 \pm 0.03$; $n = 5$) as was that for (−)-nicotine. The R-isomer of ABT 418, A-81754, was 12 times less potent ($K_i = 44$ nM) in displacing [³H]cytisine whereas (−)-nicotine was some 3-fold more potent ($K_i = 1$ nM) than ABT 418 (table 1).

In contrast to its activity at the *alpha* 4 *beta* 2 nAChR subtype labeled by [³H]cytisine (Flores *et al.*, 1992), ABT 418 was more than 3 orders of magnitude less potent (K_i value >100 μM; table 1) in displacing [¹²⁵I]α-Bgt bound to the nAChR subtype present in *Torpedo* electroplax which is similar to the neuromuscular α-BgtnAChR. ABT 418 was similarly less potent ($K_i = > 10 \mu$M) than (−)-nicotine ($K_i = 4 \mu$M) in displacing [¹²⁵I] α-Bgt binding from the α-BgtnAChR subtype present in rat brain membranes, *i.e.*, *alpha* 7 (table 1). The R-isomer of ABT 418, A-81754, also had low potency ($K_i > 10 \mu$M) at the α-BgtnAChRs present in both *Torpedo* and rat brain membranes (table 1).

ABT 418 also was examined in 38 other binding assays (table 2) and showed negligible affinity ($K_i > 10 \mu$M) for muscarinic, 5-HT$_3$ and the benzodiazepine receptors (table 2). ABT 418 had no significant effects on the binding of ligands, to other members of the ligand-gated ion channel superfamily, including γ-aminobutyric acid, benzodiazepine, N-methyl-D-aspartate, MK-801, quisqualate, kainate, L-, N- and T-calcium and potassium channel proteins; members of guanine nucleotide-binding protein-coupled receptor superfamily—adenosine A$_1$ and A$_{2a}$, *alpha*$_1$, *alpha*$_2$ and *beta* adrenergic, 5HT$_1$, 5HT$_2$, histamine H$_1$, angiotensin-II, neurokinin-1 and -2, VIP, NGF; norepinephrine, 5-HT and dopamine uptake sites and second messenger systems including adenylate cyclase, protein kinase C and IP$_3$.

⁸⁶Rb⁺ efflux from mouse thalamus. Both ABT 418 and (−)-nicotine elicited a concentration-dependent stimulation of efflux of ⁸⁶Rb⁺ from mouse thalamic synaptosomes (fig. 3) with responses of 0.7 ± 0.1 and 0.8 ± 0.1% of tissue content, respectively, for ABT 418 and (−)-nicotine at concentrations of 10 μM. The estimated EC$_{50}$ values obtained for ABT 418 (0.5 ± 0.1 μM) and (−)-nicotine (0.7 ± 0.2 μM) were similar. ABT 418 appeared to be as potent and efficacious as (−)-nicotine in stimulating ⁸⁶Rb⁺ efflux. A-81754 was not examined

in this functional model. Because agonist-induced efflux of ⁸⁶Rb⁺ from mouse thalamic synaptosomes is thought to reflect the activation of the *alpha* 4 *beta* 2 subtype of nAChR (Marks *et al.*, 1993), ABT 418 appears to be at least as efficacious as (−)-nicotine at this subtype of nAChR.

[³H]Dopamine release from rat striatal slices. ABT 418 was similar in efficacy to (−)-nicotine in stimulating the release of [³H]dopamine from rat striatal slices. However (−)-nicotine (EC$_{50}$ = 40 nM) was approximately 10-fold more potent than ABT 418 (EC$_{50}$ = 380 nM) in evoking this response (fig. 4). A-81754 had an EC$_{50}$ of greater than 1 μM in this model. The competitive nAChR antagonist, DHβE (10 μM) blocked the effects of ABT 418 (10 μM) in eliciting striatal [³H]dopamine release by 89 ± 10% ($n = 3$). DHβE (10 μM) also blocked the effects of (−)-nicotine (10 μM) on [³H]dopamine release (data not shown).

Cholinergic channel current responses in PC12 cells. The peak inward current responses to 100 μM (−)-nicotine ranged from −40 to −420 pA in 13 differentiated PC12 cells. ABT 418 (300 μM) elicited similar responses, ranging from −46 to −340 pA in another 4 cells (fig. 5). Desensitization of the response to each ligand was observed during the 5-sec application, as would be expected for nicotinic responses in these cells. Further, the nAChR channel blocker mecamylamine (10 μM) inhibited the response to ABT 418 (300 μM) by 81 ± 3% ($n = 3$).

Dose-response relationships were determined from 13 PC12 cells for (−)-nicotine, and from another four PC12 cells for ABT 418 (data not shown). The apparent EC$_{50}$ value for ABT 418 was 209 ± 76 μM whereas that for (−)-nicotine was 52 ± 4 μM, indicating that ABT 418 is about 4-fold less potent than (−)-nicotine in activating cholinergic channels in PC12 cells.

Discussion

The data presented indicate that the newly synthesized isoxazole isostere of (−)-nicotine, ABT 418, is a potent cholinergic ligand with apparent selectivity for the neuronal, but not the neuromuscular, nAChR. ABT 418 is also less potent than (−)-nicotine in displacing [¹²⁵I]α-Bgt bound to rat brain membranes (table 1). In brain, α-Bgt is thought to label a protein corresponding to the *alpha* 7 gene product (Couturier *et al.*, 1990) indicating that ABT 418 has less affinity for this subunit than does (−)-nicotine. This is important taking into consideration that approximately 50% of all nAChR in brain are of the α-BgtnAChR subtype (*i.e.*, approximately 70 fmol/mg of protein for each, see Pabreza *et al.*, 1991; Marks *et al.*, 1986). Further selectivity is demonstrated by the lack of affinity (*i.e.*, $K_i > 10$ μM) for muscarinic receptors as well as at 36 other binding sites for differing classes of receptors, enzymes, uptake sites and second messengers. The stereoselective nature of the interaction of ABT 418 with the neuronal nAChR can be demonstrated by the reduced activity of the R-isomer of ABT 418, A-81754, in both the binding and functional test systems. Ligand binding studies with [³H]ABT 418 indicate that it has a distribution in rat brain that differs from [³H]cytisine and it does not elicit the same pattern of upregulation of nicotinic receptor binding sites after chronic administration as does (−)-nicotine (Schwartz and Kellar, 1983; Wonnacott, 1990; J.P. Sullivan, personal communication). Thus, the *in vitro* phar-

TABLE 2
In vitro receptor binding selectivity of ABT 418

Receptor	Ligand	K_i
		nM
Muscarinic	[^{3}H]oxotremorine-M	>10,000
Adenosine A$_1$	[^{3}H]8-cyclopentyl-1,3-dipropylxanthine (CPX)	>10,000
Adenosine A$_{2a}$	[^{3}H]CGS 21680	>10,000
Alpha-1	[^{3}H]prazosin	>10,000
Alpha-2	[^{3}H]RX 781094	>10,000
Beta	[^{3}H]DHA	>10,000
Dopamine$_1$	(+)-[^{125}I]SCH23982	>10,000
Dopamine$_2$	[^{3}H]Spiperone	>10,000
Quisqualate	[^{3}H]AMPA	>10,000
Kainate	[^{3}H]Kainic acid	>10,000
MK-801	[^{3}H]MK-801	>10,000
NMDA	[^{3}H]CGS 19755	>10,000
PCP	[^{3}H]TCP	>10,000
Glycine, Nonstrychnine	[^{3}H]Glycine	>10,000
Sigma	[^{3}H]Di(2-tolyl)guanidine (DTG)	>10,000
Glycine, strychnine	[^{3}H]Strychnine	>10,000
GABA$_A$	[^{3}H]GABA	>10,000
GABA$_B$	[^{3}H]GABA (+50 mM isoguvacine to block GABA$_A$)	>10,000
Benzodiazepine	[^{3}H]Flunitrazepam	>10,000
Serotonin$_1$	[^{3}H]5-Hydroxytryptamine binoxalate	>10,000
Serotonin$_2$	[^{3}H]Ketanserin	>10,000
Serotonin$_3$	[^{3}H]GR65630	>10,000
Histamine	[^{3}H]Pyrilamine	>10,000
Angiotensin II, central	[^{125}I]Angiotensin II	>10,000
Neurokinin 1 (substance P)	[4,5-^{3}H-Leu]Substance P	>10,000
Neurokinin 2 (substance K)	[^{125}I]Neurokinin A	>10,000
Vasoactive intestinal peptide	[^{125}I]Vasoactive Intestinal Peptide (VIP)	>10,000
Calcium channel (type N)	[^{125}I]ω-Conotoxin	>10,000
Calcium channel (type T, L)	[^{3}H]Nitrendipine	>10,000
Chloride channel, TBOB	[^{3}H]TBOB	>10,000
Potassium channel, apamin	[^{125}I]Apamin	>10,000
Nerve growth factor	[^{125}I]Nerve growth factor	>10,000
Norepinephrine uptake	[^{3}H]Desmethylimipramine	>10,000
Serotonin uptake	[^{3}H]Citalopram	>10,000
Dopamine, cocaine site	[^{3}H]WIN 35,428	>10,000
Adenylate cyclase	[^{3}H]Forskolin	>10,000
Protein kinase C	[^{3}H]Phorbol ester dibutyrate (PDBU)	>10,000
Inositol triphosphate	[^{3}H]IP$_3$	>10,000

Fig. 3. Effect of ABT 418 and (−)-nicotine to stimulate [^{86}Rb$^+$] efflux from mouse thalamic synaptosomes. P2 fractions from mouse thalamus that had been loaded with [^{86}Rb$^+$] were exposed for 1 min to the concentrations of agonist indicated. Values are the mean ± S.E.M.; n = 3 for ABT 418 and average of two separate experiments for (−)-nicotine. ABT 418 and (−)-nicotine had estimated EC$_{50}$ values of 0.5 ± 0.1 and 0.7 ± 0.2 μM, respectively.
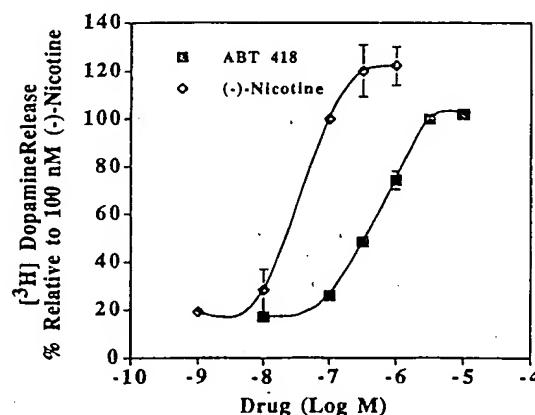

Fig. 4. Effect of ABT 418 and (−)-nicotine in stimulating the release of [^{3}H]dopamine from rat striatal slices. Values are means ± S.E.M.; ABT 418 and (−)-nicotine had EC$_{50}$s to evoke release of [^{3}H]dopamine of 380 ± 45 and 40 ± 10 nM, respectively.

macodynamic effects of ABT 418 indicate that it has an activity profile that is substantially different than (−)-nicotine.

Mechanistically, ABT 418 can activate channel currents in PC12 cells, an effect that is prevented by the noncompetitive nAChR channel blocker, mecamylamine. However, because of
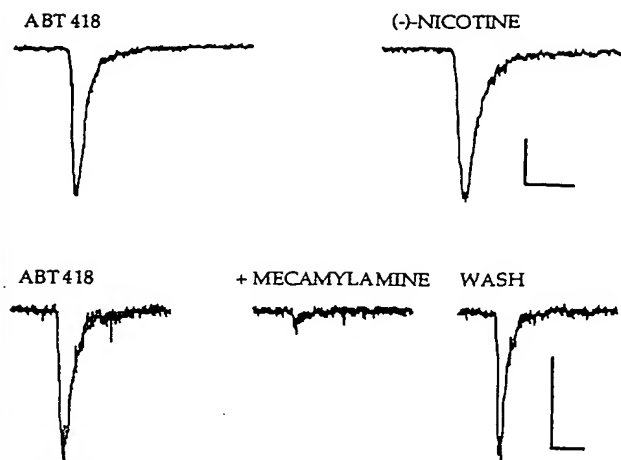
Fig. 5. Effect of ABT 418 and (−)-nicotine to elicit representative cholinergic channel currents in PC12 cells. In four nerve growth factor-differentiated PC12 cells, the inward current response to 300 μM ABT 418 (upper left trace) ranged from −46 to −340 pA. The response to 100 μM (−)-nicotine (upper right trace) similarly ranged from −40 to −420 pA in 13 other PC12 cells. The response to 300 μM ABT 418 was inhibited reversibly by 10 μM mecamylamine as shown for one cell in the lower three traces. Calibration lines represent 100 pA and 1 sec.

the noncompetitive nature of this antagonist (Gurney and Rang, 1984; Aracava et al., 1987; Rapier et al., 1990) further studies will be required to determine how ABT 418 is able to activate nAChR channel currents. The nAChR subunits associated with these cells are alpha 3, alpha 5, beta 2, beta 3 and beta 4 (Rogers et al., 1992), which are similar to those in sympathetic ganglia (Listerud et al., 1991; Sargent, 1993). ABT 418 was about 4-fold less potent than (−)-nicotine in this PC12 test system, as well as in the rat superior cervical ganglion (C. A. Briggs, personal communication), suggesting that it has less affinity for nAChR expressing the alpha 3 subunit isoform. These data would predict that in vivo ABT 418 may have fewer effects related to activation of sympathetic ganglion than does (−)-nicotine.

In another preparation that has been suggested to involve alpha 3 subunit activation (Rapier et al., 1990) ABT 418 stimulated the release of dopamine from striatal slices with an EC_{50} value of 380 nM, showing 10-fold lower potency than (−)-nicotine (EC_{50} = 40 nM). A-81754, the R-isomer of ABT 418, had an EC_{50} value of greater than 1 μM in this system. Thus, the S- and R-isomers showed an approximately 14-fold difference in affinity when measured in the binding (table 1). This is similar to the stereoselectivity reported for the enantiomers of nicotine (Wonnacott et al., 1990). In addition, the competitive nAChR antagonist DHβE (Williams and Robinson, 1984) antagonized the actions of ABT 418, suggesting that it may interact at the same site as (−)-nicotine to modulate dopamine release. It is thought that the addiction liabilities and locomotor stimulant effects of chronic exposure to compounds like (−)-nicotine are mediated by the release of dopamine elicited by the interaction of (−)-nicotine with nAChR on dopaminergic neurons (Clarke and Pert, 1985; Rapier et al., 1990; Wonnacott et al., 1990). The lower potency of ABT 418 on dopamine release compared to (−)-nicotine suggests that it may have less abuse potential than (−)-nicotine. This hypothesis is consistent with the finding that ABT 418 does not fully cross-discriminate with (−)-nicotine in rats and that it is 6-fold less potent than

(−)-nicotine to increase the firing of dopaminergic neurons in the ventral tegmental area of Tsai (Brioni et al., 1994).

Changes in rubidium flux in the mouse thalamus are thought to result from activation of the putative alpha 4 beta 2 form of the nAChR (Marks et al., 1993). In this assay, ABT 418 and (−)-nicotine appeared to have equivalent activity (estimated EC_{50} values of 500 and 700 nM, respectively). It is noteworthy that none of the "classical" nAChR agonists that have been evaluated in this assay were both as potent and as efficacious as (−)-nicotine (Marks et al., 1993). For example, the alkaloid (−)-cytisine, although twice as potent as (−)-nicotine, elicited a significantly lower maximum efflux. In contrast, acetylcholine, methylcarbamylcholine and 1,1-dimethyl-4-phenylpiperazine behaved as full agonists but were less potent than (−)-nicotine.

The concentrations of ABT 418 required to induce a functional response at the presumed alpha 4 beta 2 subtype compared with those required to displace [³H]cytisine in the binding assay differ by approximately 2 orders of magnitude. This quantitative difference may be due to the fact that stimulation of ion flux requires interaction with the receptors in their resting state whereas binding may measure the interaction of ligands with the high affinity desensitized state of the receptor (Lippiello et al., 1987).

The discrepancy in activity between ABT 418 and (−)-nicotine which is also seen in vivo (Decker et al., 1994) in regard to the hypothermia, hypolocomotor activity, seizure activity, acute lethality and emetic liability associated with (−)-nicotine suggest that ABT 418 may preferentially interact with different subtypes of the nAChR. Alternatively, ABT 418 may act as a full agonist at certain receptor subtypes, e.g., alpha 4 beta 2, while having partial agonist, or reduced activity at other receptor subtypes, an issue discussed further in regard to the in vivo actions of ABT 418 (Decker et al., 1994).

It is unclear at which site on the nAChR in brain ABT 418 binds to elicit biological responses. At the neuromuscular junction there is evidence that the alpha-1 subunits contain elements for the binding of ACh (Changeaux, 1990). For neuronal nAChRs, there has been increasing evidence that alternative "channel activator" (Pereira et al., 1993; Schrattenholz et al., 1993) and allosteric modulatory sites exist(for review see Arneric et al., 1995). Because ABT 418 has a functional profile that is different than (−)-nicotine, it is plausible that the pharmacological selectivity seen with this compound may arise from an interaction that is distinct from (−)-nicotine. Resolution of these possibilities will require more detailed pharmacological analysis of ABT 418 to be completed.

Research in the area of "nicotinic agonists" to date has been limited primarily to the biological evaluation of nicotine and other related naturally occurring alkaloids. The negative connotations associated with the recreational use of (−)-nicotine in tobacco products and the consequent negative impact on the patient health has tended to limit the research and development of nicotinic ligands as therapeutic entities. The discovery of multiple ChCA sites on nAChRs may be anticipated to renew interest in the nicotinic subclass of cholinergic receptors. ChCAs are by definition a broader pharmacological category of agents that may directly or allosterically activate one or more subtypes of the nAChR (Arneric and Williams, 1994; Lena and Changeux, 1993). A potential therapeutic outcome of developing compounds that selectively interact with nAChR is that they do not necessarily elicit a side-effect profile like (−)-

nicotine. This has conceptual parallels with the use of the term "atypical anxiolytics" (Williams, 1989) to describe compounds like CGS 20625 which, while interacting with the central BDZ receptor complex, lacked the sedative, muscle relaxant and alcohol interactive properties associated with the classical BDZ, like diazepam. ABT 418 thus may represent the first of a new class of compounds termed ChCAs that selectively activate neuronal nAChR without eliciting the dose-limiting side effects typically observed with (−)-nicotine (Arneric and Williams, 1994; Decker *et al.*, 1994; Arneric *et al.*, 1994).

Since nicotinic receptor agonists are known to improve cognitive performance in experimental animals (Haroutunian *et al.*, 1985; Elrod *et al.*, 1988; Levin, 1992; Hodges *et al.*, 1992; Decker *et al.*, 1992) and in humans (Wesnes and Warburton, 1978, 1984; Newhouse *et al.*, 1988; Rusted and Eaton-Williams, 1991; Warburton, 1992) the availability of compounds like ABT 418 may represent a novel therapeutic approach for the amelioration of the cognitive and emotional disturbances accompanying AD and other related CNS disorders. Ongoing studies at the molecular level including receptor autoradiography using [³H] ABT 418 may be anticipated to lead to the identification of the discrete molecular targets through which it produces its many actions and permit the characterization of the ChCA recognition sites responsible for the potentially beneficial actions of this novel alkaloid.

References

ALKONDON, M. AND ALBUQUERQUE, E. X.: Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. J. Pharmacol. Exp. Ther. **265**: 1455–1473, 1993.

ARACAVA, Y., DESHPANDE, S. S., SWANSON, K. L., RAPOPORT, M., WONNACOTT, S., LUNT, G. G. AND ALBUQUERQUE, E. X.: Nicotinic acetylcholine receptors in cultured neurones from the hippocampus and brain of the rat characterized by single channel recording. FEBS Lett. **222**: 63–70, 1987.

ARNERIC, S. P., SULLIVAN, J. P. AND WILLIAMS, M.: Neuronal nicotinic acetylcholine receptors: Novel targets for CNS therapeutics. *In* Psychopharmacology: Fourth Generation of Progress, ed. by F. E. Bloom and D. J. Kupfer, Raven Press, New York, in press, 1995.

ARNERIC, S. P. AND WILLIAMS, M.: Nicotinic agonists in Alzheimer's disease: Does the molecular diversity of nicotine receptors offer the opportunity for developing CNS selective cholinergic channel activators? *In* Recent Advances in the Treatment of Neurodegenerative Disorders and Cognitive Function, vol. 7, ed. by G. Racagni, N. Brunello, S. Z. Langer, pp. 58–70, Karger, Basel, 1994.

BIRDSALL, N. J. M., BURGEN, A. S. V. AND HULME, E. C.: The binding of agonists to brain muscarinic receptors. Mol. Pharmacol. **14**: 723–736, 1978.

BRIONI, J. D., KIM, D. J. B., BRODIE, M. S., DECKER, M. W. AND ARNERIC, S. P.: ABT-418: Discriminative stimulus properties and effect on ventral tegmental cell activity. Psychopharmacology, in press, 1994.

CHANGEUX, J.-P.: Functional architecture and dynamics of the nicotinic acetylcholine receptor: An allosteric ligand-gated ion channel. Fidia Res. Found. Neurosci. Found. Lect. **4**: 21–168, 1990.

CHANGEUX, J.-P., GALZI, J.-L., DEVILLERS-THIERY, A. AND BERTRAND, D.: The functional architecture of the acetylcholine nicotinic receptor explored by affinity labeling and site directed mutagenesis. Quart. Rev. Biophys. **25**: 395–432, 1992.

CLARKE, P. B. S. AND PERT, A.: Autoradiographic evidence for nicotinic receptors on nigrostriatal and and mesolimbic dopaminergic neurons. Brain Res. **348**: 355–358, 1985.

CLARKE, P. B. S., SCHWARTZ, R. D., PAUL, S. M., PERT, C. B. AND PERT, A.: Nicotinic binding in at brain: autoradiographic comparison of [³H]acetylcholine, [³H]nicotine, and [¹²⁵I]-α-bungarotoxin. J. Neurosci. **5**: 1307–1315, 1985.

COUTURIER, S., BERTRAND, D., MATTER, J.-M., HERNANDEZ, M.-C., BERTRAND, S., *et al.*: A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX. Neuron **5**: 847–856, 1990.

MICHAEL W. DECKER, JORGE D. BRIONI, JAMES P. SULLIVAN, MICHAEL J. BUCKLEY, RICHARD J. RADEK, JOANNA L. RASZKIEWICZ, CHAE HEE KANG, DAVID J. B. KIM, WILLIAM J. GIARDINA, JAMES WASICAK, DAVID S. GARVEY,

MICHAEL WILLIAMS AND STEPHEN P. ARNERIC (S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl)Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: II. *In Vivo* Characterization **270**: 319–328, 1994.

DECKER, M. W., MAJCHRZAK, M. J. AND ANDERSON, D. J.: Effects of nicotine on spatial memory deficits in rats with septal lesions. Brain Res. **572**: 281–285, 1992.

DENERIS, E. S., CONNOLLY, J., ROGERS, S. W. AND DUVOISIN, R.: Pharmacological and functional diversity of neuronal nicotinic acetylcholine receptors. Trends Pharmacol. Sci. **12**: 34–40, 1991.

ELROD, K., BUCCAFUSCO, J. J. AND JACKSON, W. J.: Nicotine enhances delayed matching-to-sample performance in primates. Life Sci. **43**: 277–287, 1988.

ENNA, S. J. AND SNYDER, S. H.: Properties of γ-aminobutyric acid receptor binding in rat brain synaptic membrane fractions. Brain Res. **100**: 81–87, 1977.

FALCH, E., JACOBSEN, P., KROGSGAARD-LARSEN, P. AND CURTIS, D. R.: GABA-mimetic activity and effects on diazepam binding of aminosulphonic acids structurally related to piperidine-4-sulphonic acid. J. Neurochem. **44**: 68–75, 1985.

FENWICK, E. M., MARTY, A. AND NEHER, E.: A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. J. Physiol. (Lond.) **331**: 577–597, 1982.

FLORES, C. M., ROGERS, S. W., PABREZA, L. A., WOLFE, B. AND KELLAR, K. J.: A subtype of nicotinic cholinergic receptor is composed of α4 and β2 subunits and is upregulated by chronic nicotine treatment. Mol. Pharmacol. **41**: 31–37, 1992.

GARVEY, D. S., WASICAK, J., DECKER, M. W., BRIONI, J. D., BUCKLEY, M. J., SULLIVAN, J. P., CARRERA, G. M., HOLLADAY, M. H., ARNERIC, S. P. AND WILLIAMS, M.: Novel isoxazoles which interact with brain cholinergic channel receptors that have intrinsic cognitive enhancing and anxiolytic activities. J. Med. Chem. **37**: 1055–1059, 1994.

GRADY, S., MARKS, M. J., WONNACOTT, S. AND COLLINS, A. C.: Characterization of nicotinic receptor mediated [3H]dopamine release from synaptosomes prepared from mouse striatum. J. Neurochem. **59**: 848–856, 1992.

GURNEY, A. M. AND RANG, H. P.: The channel blocking action of methonium compounds on rat submandibular ganglion cells. Br. J. Pharmacol. **82**: 623–642, 1984.

HAROUTUNIAN, V., BARNES, E. AND DAVIS, K. L.: Cholinergic modulation of memory in rats. Psychopharmacology **87**: 266–271, 1985.

HODGES, H., SINDEN, J., TURNER, J. J., NETTO, C. A., SOWINSKI, P. AND GRAY, J. A.: Nicotine as a tool to characterize the role of the basal forebrain cholinergic projection system in cognition. *In* The Biology of Nicotine: Current Research Issues, ed. by P. M. Lippiello, A. C. Collins, J. A. Gray and J. H. Robinson, pp. 157–182. New York, Raven Press, 1992.

HOYER, D. AND NEIJT, H. C.: Identification of serotonin 5-HT₃ recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. Mol. Pharmacol. **33**: 303–309, 1988.

JONES, G. M. M., SAHAKIAN, B. J., LEVY, R., WARBURTON, D. M. AND GRAY, J. A.: Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology **108**: 485–494, 1992.

LENA, C. AND CHANGEUX, J.-P.: Allosteric modulation of the nicotinic acetylcholine receptor. Trends Pharmacol. Sci. **16**: 181–186, 1993.

LEVIN, E. D.: Nicotinic systems in cognitive function. Psychopharmacology **108**: 417–431, 1992.

LIPPIELLO, P. M., SEARS, S. B. AND FERNANDES, K. G.: Kinetics and mechanism of action of L-[³H] nicotine binding to putative high affinity receptor sites in rat brain. Mol. Pharmacol. **31**: 392–400, 1987.

LISTERUD, M., BRUSSAARD, A. B., DEVAY, P., COLMAN, D. R. AND ROLE, L. W.: Functional contribution of neuronal AChR subunits revealed by antisense oligonucleotides. Science (Washington, DC) **254**: 1518–1521, 1991.

LUETJE, C. W. AND PATRICK, J.: Both α- and β-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. J. Neurosci. **11**: 837–845, 1991.

MARKS, M. J., FARNHAM, D. A., GRADY, S. R. AND COLLINS, A. C.: Nicotinic receptor function determined by stimulation of nibidium efflux from mouse brain synaptosomes. J. Pharmacol. Exp. Ther. **264**: 542–552, 1993.

MARKS, M. J., STITZEL, J. A., ROMM, E., WEHNER, J. M. AND COLLINS, A. C.: Nicotinic binding sites in rat and mouse brain: comparison of acetylcholine, nicotine and α-bungarotoxin. J. Pharmacol. Exp. Ther. **30**: 427–436, 1986.

MISHINA, M., TAKAI, T., IMOTO, K., NODA, M., TAKAHASHI, T., NUMA, S., METHFESSEL, C. AND SAKMANN, B.: Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature (Lond.) **321**: 406–411, 1986.

MULLE, C., VIDAL, C., BENOIT, P. AND CHANGEUX, J.-P.: Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo-interpeduncular system. J. Neurosci. **11**: 2588–2597, 1991.

NEWHOUSE, P. A., SUNDERLAND, T., TARIOT, P. N., BLUMHARDT, C. L. WEINGARTNER, H., MELLOW, A. AND MURPHEY, D. L.: Intravenous nicotine in Alzheimer's disease: A pilot study. Psychopharmacology **95**: 171–175, 1988.

PABREZA, L. A., DHAWAN, S. AND KELLAR, K. J.: [³H]Cytisine binding to nicotinic cholinergic receptors in brain. Mol. Pharmacol. **39**: 9–12, 1991.

PAPKE, R. L.: The kinetic properties of neuronal nicotinic receptors: Genetic basis of functional diversity. Prog. Neurobiol. **41**: 509–531, 1993.

PEREIRA, E. F. R., REINHARDT-MAELICKE, S., SCHRATTENHOLZ, A., MAELICKE,

A. AND ALBUQUERQUE, E. X.: Identification and functional characterization of a new agonist site on nicotinic acetylcholine receptors of cultured hippocampal neurons. J. Pharmacol. Exp. Ther. 265: 1474–1491, 1993.

RAPIER, C., LUNT, G. G. AND WONNACOTT, S.: Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. J. Neurochem. 50: 1123–1130, 1990.

ROGERS, S. W., MANDELZYS, A., DENERIS, E. S., COOPER, E. AND HEINEMANN, S.: The expression of nicotinic acetylcholine receptors by PC12 cells treated with NGF. J. Neurosci. 12: 4611–4623, 1992.

RUSTED, J. AND EATON-WILLIAMS, P.: Distinguishing between attentional and amnestic effects in information processing: The separate and combined effects of scopolamine and nicotine on verbal free recall. Psychopharmacology 104: 363–366, 1991.

SAHAKIAN, B., JONES, G., LEVY, R., GRAY, J. AND WARBURTON, D.: The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. Br. J. of Psychiatry 154: 797–800, 1989.

SARGENT, P. B.: The diversity of neuronal nicotinic acetylcholine receptors. Ann. Rev. Neurosci. 16: 403–443, 1993.

SCHOEPFER, R., CONROY, W. G., WHITING, P., GORE, M. AND LINDSTROM, J.: Brain α-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. Neuron 5: 35–48, 1990.

SCHRATTENHOLZ, A., COBAN, T., SCHRODER, B., OKONJO, K. O., KUHLMANN, J., PEREIRA, E. F., ALBUQUERQUE, E. X. AND MAELICKE, A.: Biochemical characterization of a novel channel activating site on nicotinic acetylcholine receptors. J. Receptor Res. 13: 393–412, 1993.

SCHWARTZ, R. D. AND KELLAR, K. J.: Nicotinic cholinergic binding sites in the brain: In vivo regulation. Science (Washington, DC) 220: 214–216, 1983.

SEGUELA, P., WADICHE, J., DINELEY-MILLER, K., DANI, J. A. AND PATRICK, J. W.: Molecular cloning, functional properties and distribution of rat brain α7. A nicotinic cation channel highly permeable to calcium. J. Neurosci. 13: 596–604, 1993.

WARBURTON, D. M.: Nicotine as a cognitive enhancer. Prog. Neuropsychophar-macol. Biol. Psychiatry 16: 181–191, 1992.

WESNES, K. AND WARBURTON, D. M.: The effects of cigarette smoking and nicotine tablets upon human attention. In Smoking Behavior: Physiological and Psychological Influences, ed. by R. E. Thornton, pp. 131–147, Churchill-Livingston, London, 1978.

WESNES, K. AND WARBURTON, D. M.: Effects of scopolamine and nicotine on human rapid information processing performance. Psychopharmacology 82: 147–150, 1984.

WHITING, P. AND LINDSTROM, J.: Pharmacological properties of immuno-iso-lated neuronal nicotinic receptors. J. Neurosci. 6: 3061–3069, 1986.

WHITING, P. AND LINDSTROM, J.: Characterization of bovine and human neu-ronal nicotinic acetylcholine receptors using monoclonal antibodies. J. Neu-rosci. 8: 3395–3404, 1988.

WILLIAMS, M.: Antianxiety agents: A historical perspective. In Current and Future Trends in Anticonvulsant, Anxiety and Stroke Therapy, ed. by B. S. Meldrum and M. Williams, Alan R. Liss, New York, pp. 477–482, 1989.

WILLIAMS M. AND ROBINSON, J. L.: Binding of the nicotinic cholinergic antag-onist, dihydro-beta-erythroidine (DBE) to rat brain tissue. J. Neurosci. 4: 2906–2911, 1984.

WONNACOTT, S.: α-Bungarotoxin binds of low-affinity binding sites in rat brain. J. Neurochem. 47: 1706–1712, 1986.

WONNACOTT, S.: The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends Pharmacol. Sci. 11: 216–219, 1990.

WONNACOTT, S., IRONS, J., RAPIER, C., THORNE, B. AND LUNT, G. G.: Presyn-aptic modulation of transmitter release by nicotinic receptors. In Progress in Brain Research, ed. by A. Nordberg, K. Fuxe, B. Holmstedt and A. Sundwall, pp. 157–163, Amsterdam, Elsevier, 1990.

ZIFA, E. AND FILLION, G.: 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44: 401–458, 1992.

(S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl)Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: II. In Vivo Characterization

MICHAEL W. DECKER, JORGE D. BRIONI, JAMES P. SULLIVAN, MICHAEL J. BUCKLEY, RICHARD J. RADEK, JOANNA L. RASZKIEWICZ, CHAE HEE KANG, DAVID J. B. KIM, WILLIAM J. GIARDINA, JAMES T. WASICAK, DAVID S. GARVEY, MICHAEL WILLIAMS and STEPHEN P. ARNERIC

Neuroscience Discovery (M.W.D., J.D.B., J.P.S., M.J.B., R.J.R., J.L.R., C.H.K., D.J.B.K., J.W., O.S.G., M.W., S.P.A.) and Drug Design and Delivery (W.J.G.), Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois

Accepted for publication March 3, 1994

ABSTRACT

(S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT 418), an isoxazole analog of (−)-nicotine, is a potent agonist at the alpha-4/beta-2 subtype of neuronal nicotinic acetylcholine receptor (nAChR) that exists in mammalian brain (Arneric et al., 1994). Compared to (−)-nicotine, ABT 418 has reduced potency to interact with the subunit isoforms of nAChR found in sympathetic ganglia, and it does not compete for alpha-bungarotoxin binding sites in brain or at the neuromuscular junction. ABT 418 [minimum effective dose (MED), 0.062 μmol/kg i.p.] was 10-fold more potent in improving retention of avoidance learning in normal mice than (−)-nicotine, whereas the (R)-enantiomer of ABT 418, A-81754, was inactive. The memory-enhancing effect of ABT 418 was prevented by the nAChR channel blocker, mecamylamine. In the elevated plus-maze model of anxiety, ABT 418 (MED, 0.19 μmol/kg i.p.) increased open-arm exploration in mice, as previously shown for (−)-nicotine (MED, 0.62 μmol/kg i.p.). A-81754, did not have anxiolytic-like effects in this test. Unlike the classical anxiolytic, diazepam, ABT 418 did not impair rotorod performance in the dose range where beneficial effects occurred. In rats, ABT 418 (MED, 0.002 μmol/kg i.v.) was remarkably potent in enhancing basal forebrain-elicited increases in cortical cerebral blood flow, whereas resting cerebral blood flow was unaffected. Free running cortical electroencephalography in rats was unaffected by ABT 418 at a dose of 1.9 μmol/kg i.p., whereas the same dose of (−)-nicotine caused cortical activation (decreased power in the 1–13 Hz range and increased power in the 25–50 Hz range). Whereas ABT 418 was approximately 3- to 10-fold more potent than (−)-nicotine in memory enhancement and anxiolytic test paradigms, the compound had less emetic liability in dogs as compared to (−)-nicotine, and was less potent than (−)-nicotine in eliciting hypothermia, seizures, death and reduction of locomotor activity in mice. The measured pharmacokinetic or brain disposition properties of ABT 418 in rats did not account for the observed enhancement in efficacy with reduced toxicity as compared to (−)-nicotine. The potent cognitive-enhancing and anxiolytic properties obtained for ABT 418 in animal models without eliciting significant side effects suggest that this ligand is a selective activator of cholinergic channel-mediated behaviors. Thus, ABT 418 may represent a novel, safe and effective treatment of the cognitive and emotional dysfunctions associated with Alzheimer's disease.

AD is associated with a loss of learning and memory abilities, attentional deficits, anxiety, agitation and depression (Perry et al., 1978). Other impaired brain functions include reductions in cerebral blood flow, cerebral glucose utilization and abnormal EEG (Dastur, 1985; Petit et al., 1993). Topographically these impaired brain functions correspond to the loss of the cholinergic innervation arising from the basal forebrain (Coyle et al., 1983) and to substantial reductions in neuronal nAChRs (Whitehouse et al., 1981, 1986).

Although the prevailing dogma suggests that muscarinic cholinergic receptors mediate the primary effects of central cholinergic transmission on cognitive performance (Bartus et al., 1982, 1985) and cerebral vasodilation (Lee, 1982; Pinard, 1989; Hamel and Estrada, 1989), replacement therapy targeting muscarinic receptors has not been a fruitful approach to the amelioration of AD symptomatology (Arneric and Williams, 1993; Williams, 1993). However, pilot clinical data indicate that acutely administered (−)-nicotine, the prototypic agonist for nAChR, may be beneficial for the treatment of the deficits in attention and information processing associated with AD (Newhouse et al., 1988; Sahakian et al., 1989; Jones et al., 1992).

Extensive evidence exists to indicate that activation of nAChR improves cognitive performance and improves cerebral functions in experimental animals and normal humans. (−)-Nicotine enhances cognitive function in normal rats (Levin *et al.*, 1990; Levin, 1992) and attenuates memory deficits produced by destruction of cholinergic input to the cortex and hippocampus (Tilson *et al.*, 1988; Decker *et al.*, 1992; Hodges *et al.*, 1992), an effect shared by some other nAChR agonists (Decker *et al.*, 1993; Meyer *et al.*, 1994). In addition, (−)-nicotine improves short-term memory performance in both young and aged monkeys (Elrod *et al.*, 1988; Buccafusco and Jackson, 1991). The involvement of nicotinic neurotransmission in cognitive function processes is further substantiated by observed deficits in cognitive performance after administration of mecamylamine, a nAChR channel blocker, to rodents (Oliverio, 1966; Levin *et al.*, 1987; Riekkinen *et al.*, 1990; Decker and Majchrzak, 1992), monkeys (Elrod *et al.*, 1988) and humans (Newhouse *et al.*, 1992). Moreover, the characteristic cortical cerebral blood flow abnormality associated with AD reflects nAChR deficits. Specifically, it has been demonstrated that mecamylamine, but not the muscarinic antagonist, scopolamine, reduces resting cortical cerebral blood flow in the parietotemporal cortex of humans (Gitelman and Prohovnik, 1992), the area most consistently implicated in functional brain imaging of AD patients (Prohovnik *et al.*, 1988; Risberg *et al.*, 1990; Geaney *et al.*, 1990; Heiss *et al.*, 1990). Reduced nicotinically mediated cerebral blood flow responses would, thus, be consistent with the loss of nAChR reported in several cortical regions using various labeling techniques (Whitehouse *et al.*, 1986; Araujo *et al.*, 1988; Schroder *et al.*, 1991; Aubert *et al.*, 1992), as well as a report suggesting a loss of basal forebrain nAChR population in AD (Shimohama *et al.*, 1986).

(−)-Nicotine, however, has limited utility as a therapeutic agent for AD because of its dose-limiting side effects in humans, which are primarily gastrointestinal (*e.g.*, nausea, abdominal pain) and cardiac (*e.g.*, increased catecholamine released resulting in tachycardia, peripheral vasoconstriction and elevated blood pressure) in nature. In an aged patient population these latter effects may result in more serious complications (Benowitz, 1992), especially in patients with pre-existing arrhythmias or angina pectoris. Compounds that selectively interact with subtypes of nAChR to normalize CNS functions modulated by this receptor superfamily may, therefore, offer the potential for more effective therapeutic agents.

ABT 418 is a novel bioisostere of (−)-nicotine (Arneric' *et al.*, 1994; Garvey *et al.*, 1994) that selectively activates mammalian brain nAChRs in a manner that suggests interactions with a subpopulation of non-*alpha*-bungarotoxin sensitive nAChRs. A summary of some of the *in vitro* findings with this compound from Arneric' *et al.* (1994) is presented in table 1. The present study demonstrates *in vivo* that ABT 418, while sharing many of the positive CNS attributes of (−)-nicotine, has a reduced propensity to elicit the side effects that limit the potential of (−)-nicotine for the safe treatment of AD.

Materials and Methods

All animal studies were conducted in accord with American Association for the Accreditation of Laboratory Animal Care (AAALAC) procedures as approved by the Institutional Animal Care and Use Committee at Abbott Laboratories.

TABLE 1

Overview of the *In vitro* pharmacological properties of ABT 418 and the (*R*)-enantiomer, A-81754, compared to (−)-nicotine

Assay Procedure	ABT 418 (S-form)	A-81754 (R-form)	(−)-Nicotine (S-form)
nAChR binding (K_i, nM)			
[^{3}H]cytisine	3 ± 0.4	44 ± 12	1 ± 0.1
[^{125}I]α-bungarotoxin	>10,000	>10,000	4000 ± 800
PC12 cell current activation (EC$_{50}$, µM)	209 ± 76	ND[a]	52 ± 4
^{86}Rb$^+$ efflux from thalamic synaptosomes (estimated EC$_{50}$, µM)	0.5 ± 0.1	ND[a]	0.7 ± 0.2
[^{3}H]Dopamine release from striatal slices (EC$_{50}$, µM)	0.38 ± 0.05	>1	0.04 ± 0.01

[a] ND, not determined.

Compounds

ABT 418 and its (*R*)-enantiomer, A-81754, were synthesized as described (Garvey *et al.*, 1994). (−)-Nicotine di-(+)-tartrate salt, (+)-nicotine di-*p*-toluoyltartrate salt, mecamylamine hydrochloride, diazepam and urethane were obtained from Sigma Chemical Company (St. Louis, MO). Morphine sulfate was obtained from Mallinckrodt (St. Louis, MO). These compounds were dissolved in sterile 0.9% saline and injected in a volume of 10 ml/kg for mice and 1 ml/kg for rats. Fresh solutions were prepared each day. Curare injectable was purchased from Abbott Laboratories (Abbott Park, IL). Halothane was purchased from Halocarbon Laboratories, Inc. (North Augusta, SC).

Behavioral Studies

Male, CD-1 mice (Charles River, Portage, MI) weighing approximately 30 g were used in the behavioral experiments. The mice were housed 14 to a cage in a climate-controlled environment with free access to food and water. A 12:12-hr light/dark cycle (lights on at 6:00 A.M.) was used with testing conducted during the light portion of the cycle.

Inhibitory (passive) avoidance. Inhibitory avoidance training was conducted using an automated avoidance training system (Gemini, San Diego Instruments, San Diego, CA). ABT 418 and A-81754 were administered i.p. 15 min before the beginning of the training session. When used, the nAChR channel blocker, mecamylamine, or saline was administered 5 min before the ABT 418. Training was initiated by placing the mouse in a 13 × 14 × 13 (L x W x H) cm, brightly lit chamber. After a delay of 10 sec, a guillotine door leading to a larger (21 × 25 × 17 cm), darkened chamber automatically opened. When the mouse crossed completely into the dark chamber, the door was closed and a scrambled, constant current (0.3 mA) footshock was delivered through a grid floor for 2 sec. After the termination of the footshock the mouse was removed from the apparatus and returned to its home cage. Retention of the training experience was assessed 24 hr later by placing the mouse in the brightly lit compartment and measuring its latency to enter the dark compartment. On this retention test day, the trial was terminated after 180 sec and animals not crossing into the dark chamber before the end of the session were assigned a latency score of 180 sec. The latency to enter the dark chamber during this retention test session was used as the index of memory for the training experience. Inhibitory avoidance data were not distributed normally, so these results were evaluated using the nonparametric Mann-Whitney U test.

Elevated-plus-maze. Anxiolytic-like activity was evaluated using the elevated plus-maze, a pharmacologically validated model (Brioni *et al.*, 1993; Pellow *et al.*, 1985) according to procedures previously described (Brioni *et al.*, 1993). The elevated plus-maze was custom made of gray Plexiglas and consisted of two open arms (17 × 8 cm) and two enclosed arms (17 × 8 × 15 cm) extending from a central platform (8 × 8 cm) mounted on a plywood base raised 39 cm above the floor. Light

levels on the open and enclosed arms were similar. A video camera was mounted on the ceiling above the apparatus and the experiments were taped for later behavioral evaluation. At the beginning of the experiment, mice were placed in the center of the maze and the following variables were scored: 1) the time spent in the open arms (a measure of anxiolytic-like activity) and 2) the number of entries into the four arms (a measure of general activity). An arm entry was defined as the entry of all four feet of the animal into one arm. The test lasted 5 min. All animals used were naive to the apparatus.

Body temperature, locomotor activity and rotorod performance. In these experiments, both body temperature and open field locomotor activity were measured in the same mice. Beginning 4 min after an i.p. injection of the nAChR ligands (ABT 418, (−)-nicotine or their enantiomers), horizontal activity counts were recorded for 15 min in a 41×41 cm. open field using Digiscan activity monitors (Omnitech Electronics, Columbus, Ohio). In some experiments, the nAChR channel blocker, mecamylamine, or saline was administered 4 min before the agonist. Body temperature was measured using a rectal probe inserted 3 cm into the rectum (YSI TeleThermometer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Body temperature was determined at two time points: approximately 20 min (immediately after the mice were removed from the open field) and 60 min after injection of the agonist. Temperature and locomotor activity data were analyzed using ANOVA (SuperANOVA, Abacus Concepts, Inc., Berkeley, CA), with post-hoc pairwise comparisons evaluated using Fisher's protected least significant difference test.

Motor coordination was assessed in mice using an accelerated rotorod test procedure using previously published methods (Jones and Roberts, 1967). Diazepam (3.5, 10.5 and 35.5 μmol/kg i.p.) served as a CNS reference depressant.

Antinociceptive activity. The effects of ABT 418 on nociception were determined using the Woolfe-MacDonald hot-plate test (Woolfe and MacDonald, 1944). The latency to licking or shaking of the hind paw was measured after mice were placed on the hot plate (56.5°C) at 15, 30 and 60 min after dosing. Morphine sulphate (14.7 μmol/kg) served as a reference standard.

Seizure and anticonvulsant activity. Seizure liability was determined both in the presence and absence of the nicotinic receptor antagonist, mecamylamine. Five min after administration of mecamylamine (15 μmol/kg) or saline, (−)-nicotine or ABT 418 was administered i.p. and animals were observed for gross behavioral signs of seizure activity. Appropriate doses were selected on the basis of pilot experiments and seven animals were included in each group. Seizure ED_{50} values were calculated for each treatment condition using the method of Litchfield and Wilcoxon using the PHARM/PCS program (MicroComputer Specialists, Philadelphia, PA). Single doses with 100 and 0% seizures were used in the calculations of the ED_{50} values.

Anticonvulsant activity of ABT 418 was assessed by pretreating mice 30 min before the i.v. administration of pentylenetetrazol (0.5% solution infused at 0.3 ml/min). Diazepam (35.2 μmol/kg i.p.) served as a reference compound.

Lethality. Lethality was assessed using a variation of the approximate lethal dose procedure. After initial range-finding experiments, approximate lethal dose values were determined in groups of seven mice, each mouse in a group receiving one of seven doses separated by 10-μmol/kg increments. In each group of seven mice, the lowest dose at which an animal died within 24 hr of injection was designated as the approximate lethal dose for that group. Four such groups were used to determine the mean approximate lethal dose for ABT 418 and (−)-nicotine and three groups were used to determine the mean approximate lethal dose for A-81754.

Pharmacokinetic Analysis

ABT 418 and (−)-nicotine were extracted from rat plasma and brain using a mixture of 0.1 ml of biological tissue to 1.0 ml of buffer basified with 0.1 to 0.6 ml of 0.5 M K_2CO_3. This mixture was extracted with 5 to 8 ml of hexane/ethyl acetate (1:1) by vortexing and centrifuging at 3,000 rpm for 15 min at 18°C. The organic phase was back extracted

with 0.3 ml of 0.02 N HCl by vortexing and centrifuging as indicated above. To prepare brain tissue homogenates the brain was homogenized in $5 \times$ weight volume of cold 1 N perchloric acid and centrifuged at 18,500 rpm for 20 min at 4°C. The supernatant was adjusted to pH 10.6 with 2 M K_2CO_3 and treated as described above. Traces of organic solvent in the acid extract from plasma and brain was removed in a fume hood for 1.5 hr at room temperature without N_2 blowing or heating before analyses by high-performance liquid chromatography.

The analytical procedure consisted of injecting 5 to 50 μl of extract into a high performance liquid chromatographic system (Hewlett Packard model 1050, Naperville, IL) fitted with a C_{18} reverse phase column (15×0.46 cm internal diameter; ODS-AQ, 5-μm spherical particles, YMC) and a Coulochem II electrochemical detector (ESA, Bedford, MA). The electrochemical detector was fitted with a conditioning cell (model 5021) and an analytical cell (model 5010) interfaced with a Rainin integrator (Rainin Inc, Woburn, MA). Chromotography was accomplished isocratically at a flow rate of 1 ml/min using a mobile phase for ABT 418 consisting of acetonitrile, methanol and 50 mM K-PO_4 and 10 mM tetramethyl ammonium hydroxide (18:4:78), pH 6.8; whereas the mobile phase for (−)-nicotine consisted of acetonitrile, methanol and 20 mM K-PO_4 (10:10:80) with pH adjusted to 6.3 with tetramethyl ammonium hydroxide. Corresponding calibration curves were run and sample values calculated using the Rainin Dynamax program. The sensitivity of the method was 0.5 ng/ml in plasma and 1.0 ng/g in brain tissue.

Cerebral Blood Flow Measurements

Methods for surgical preparation of rats for electrical stimulation of brain and measurement of cerebral blood flow were described previously in detail (Linville and Arneric, 1991) and are summarized below. Studies were conducted on male Sprague-Dawley rats. Animals were anesthetized with urethane (1.5 g/kg) after induction with halothane (3.5% balance O_2) delivered through a nose mask. Thin-wall vinyl catheters (outer diameter, 0.03 inch) were placed in the left femoral vein and artery for drug administration and monitoring of cardiovascular parameters, respectively, and the trachea was cannulated. Animals were subsequently placed in a Kopf stereotaxic frame with the head positioned so that the floor of the IVth ventricle was horizontal (incisor bar position, −11 mm), ventilated at 80 cpm (Harvard Apparatus, model 680, Harvard Instrument Inc., South Natick, MA) with 100% O_2 and paralyzed with d-tubocurarine (0.6 mg/kg/hr i.m.).

The procedure for eliciting an increased cortical cerebral blood flow response requires the stereotaxic placement of a stainless steel concentric bipolar electrode into the basal forebrain. Cerebrovascular responsiveness, as measured by laser-Doppler flowmetry, was used to localize the most active site of the basal forebrain with 10-sec trains of 2-msec duration pulses, at a frequency of 50 Hz and intensity of 100 μA. Briefly, the laser-Doppler flowmetry probe (0.8 mm diameter) was stereotaxically positioned within a restricted cortical region (0.3 ± 0.3 mm anterior and 1.8 ± 0.5 mm lateral to Bregma). The laser-Doppler flowmetry monitor (model PF-3, Perimed, Stockholm, Sweden) does not display actual perfusion units. Therefore, for the experiments discussed, these values are treated as relative perfusion units and only used to determine the percentage of changes in cerebral blood flow.

Experimental procedure. When stimulus-induced cerebral blood flow increases of approximately 100% were repeatedly obtained in the absence of significant changes in arterial pressure (<10 mm Hg), and when the perfusion rate was stable in the absence of basal forebrain stimulation, the experimental testing was initiated. A frequency-response curve was generated at 12.5, 25 and 50 Hz, (100 μA constant current) stimulations. Subsequently, the animal was infused with the cholinergic agents i.v. and frequency-response curves using the identical stimulation parameters were generated during the 3-to-15-min postinjection period. Compounds were administered in order of increasing concentration, and the order of stimulus frequency presentation was counterbalanced between animals during the postinjection period so as not to bias frequency-graded responses by the time from administration of compound. For the experiments examining the effects of drugs on

the resting or basal forebrain-elicited cortical cerebral blood flow response the data were calculated as the percentage of changes followed by log-transformed before ANOVA, because comparing the percentage of changes across treatment groups cannot be assumed to follow a normal distribution. Two-way repeated measures ANOVA were used, both drug dose and stimulation frequency being within-subjects measures. Post-hoc analyses consisted of multiple paired comparisons (t tests) at each frequency using a Bonferroni correction of the statistical criterion dependent upon the number of comparisons made. The criterion of statistical analysis was $P < .05$

EEG

Six-month-old male Wistar rats (CAMM Research, Wayne, NJ) were surgically implanted with epidural recording electrodes according to methods described previously (Radek, 1993). The effects of (−)-nicotine or ABT-418 on the EEG of conscious, unrestrained rats were determined for recordings over the left parietal cortex (posterior, −2.0; lateral, 3.0 in mm from bregma). Amplified EEG signals (filtered at 1 and 100 Hz) at a digital sampling rate of 256 HZ were recorded using a computer-based acquisition and analysis system (Brainwave Systems, Broomfield, CO). The EEG was evaluated by fast Fourier transform power analysis specific frequency bands. Two recordings were made from each rat, one after administration of saline and the other after administration of either (−)-nicotine or ABT 418 (1.9 μmol/kg i.p. for each compound). The order of administration saline and the test compound was counterbalanced.

Emetic Liability

Emetic liability was assessed in male or female beagle dogs (10–20 kg). Conscious dogs were gently restrained and drug (500 nmol/kg dissolved in 10 ml of sterile saline) was slowly administered intravenously over a 2-min period. The presence or absence of emesis occurring over an 8-hr period was recorded.

Results

Mouse Behavioral Studies

Inhibitory (passive) avoidance. ABT 418 enhanced inhibitory avoidance retention test performance at a dose of 0.062 μmol/kg (fig. 1; $P < .05$, two-tailed Mann-Whitney U test). Doses a log unit higher or lower than this effective dose did not significantly improve performance, resulting in the inverted U-shaped dose-response curve typically reported for memory-enhancing agents (Flood et al., 1981; Gold, 1989). The effects
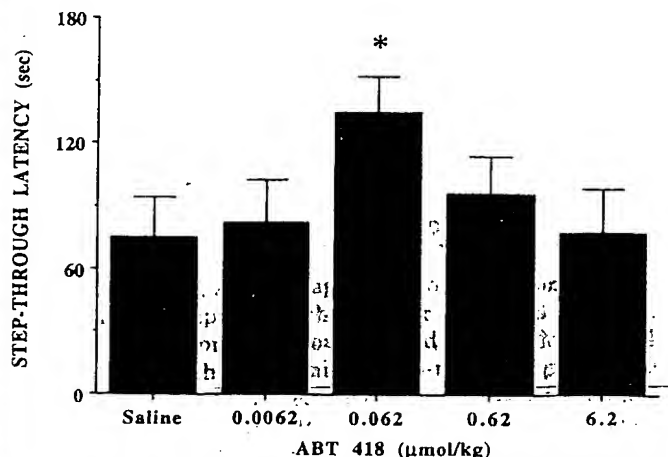
of ABT 418 and A-81754 were compared in a related experiment. ABT 418 was effective at 0.062 μmol/kg ($P = .054$, two-tailed Mann-Whitney U test), whereas the enantiomer, A-81754, did not affect performance at this dose or at a dose 10 times higher (table 2). Pretreatment with mecamylamine, a nAChR channel blocker, at a dose which did not by itself affect performance (5 μmol/kg), prevented the memory-enhancing effect of ABT 418 (fig. 2).

Elevated plus-maze. ABT 418 (i.p.) induced a significant increase in the time spent in the open arms in mice after i.p. injections ($F_{(4,69)} = 2.9$; $P < .05$). The anxiolytic-like effect of ABT 418 was induced at the 0.19 and 0.62 μmol/kg doses (fig. 3). In contrast, these doses of ABT 418 did not affect the general activity of the animals, as measured by total arm entries ($F_{(4,69)} = 0.3$; N.S., data not shown). The (R)-enantiomer of ABT 418, A-81754, did not produce an anxiolytic-like effect over the dose range (0.019–6.2 μmol/kg i.p.) tested ($F_{(6,51)} = 1.26$; N.S., data not shown).

Body temperature and locomotor activity and rotorod performance. Body temperature and open field locomotor activity were both markedly reduced by (−)-nicotine (table 3). In contrast, (+)-nicotine had no apparent effect on body temperature and only a mild effect on locomotor activity (table 3). ABT 418 and A-81754 both reduced body temperature (table 3), although they were considerably less potent than (−)-nic-

TABLE 2
Comparison of the effects of ABT 418 and its (*R*)-enantiomer, A-81754, on inhibitory avoidance performance in mice
Values are mean ± S.E.M.

Dose	n per Group	Latency to Cross
μmol/kg		*sec*
Saline	14	63.9 ± 17.5
ABT 418		
0.019	15	68.5 ± 17.8
0.062	9	114.1 ± 11.9*
0.19	10	64.2 ± 18.5
0.62	10	83.2 ± 18.1
A-81754		
0.019	12	93.4 ± 18.2
0.062	11	68.2 ± 17.2
0.19	10	60.1 ± 12.5
0.62	12	68.8 ± 15.7

* Different from control ($P = 0.054$; two-tail Mann-Whitney U test).

Fig. 1. Effect of ABT 418 on retention of inhibitory avoidance performance in mice. Values are mean ± S.E.M.; $n = 12$. Different from saline; $P < .05$.
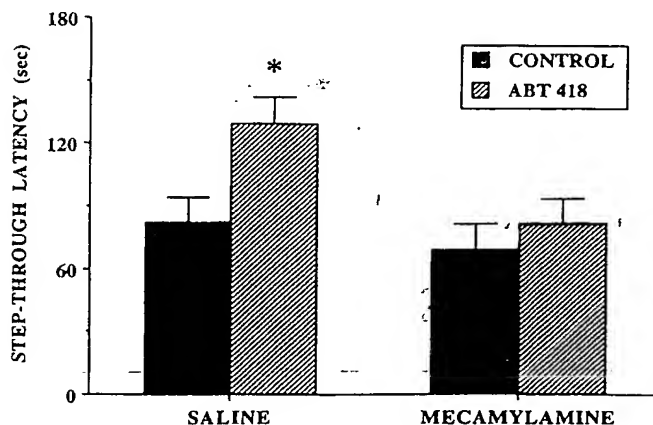

Fig. 2. Effect of pretreatment with mecamylamine (5 μmol/kg) on ABT 418-induced enhancement of inhibitory avoidance performance in mice. Values are mean ± S.E.M.; $n = 27$–28. Different from all other groups; $P < .02$.
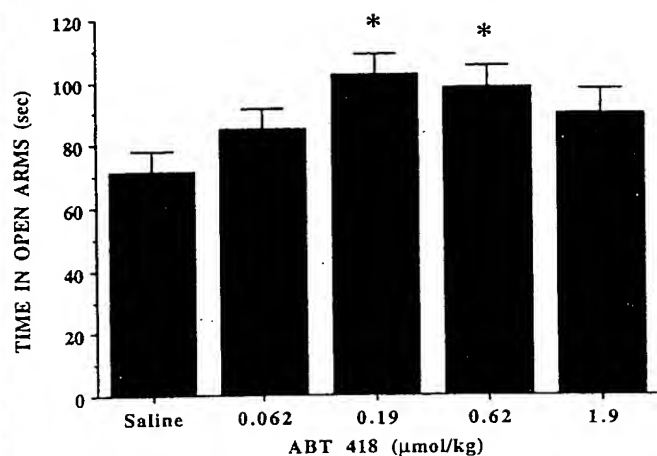
Fig. 3. Anxiolytic-like effect of ABT 418 in the mouse elevated plus-maze. Values are mean ± S.E.M.; n = 14 to 16. *Different from saline; P < .05.

TABLE 3

Effect of (−)-nicotine, (+)-nicotine, ABT 418 and A-81754 on locomotor activity and body temperature

Values shown are the mean ± S.E.M.; n = 7 to 8 per group. For the (−)- and (+)-nicotine activity data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,46)}$ = 12.57; P < .0001] and Fisher PLSD post-hoc test (* P < .05; ** P < .01; *** P < .001; **** P < .0001). For the ABT 418 and A-81754 activity data, the differences from saline control evaluated by one-way ANOVA [$F_{(6,48)}$ = 3.53; P < .01] and Fisher PLSD post-hoc test (* P < .05; ** P < .01). For (−)- and (+)-nicotine temperature data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,46)}$ = 125.92; P < .0001 for 20 min and $F_{(6,46)}$ = 2.54; P < .05 for 60 min] and Fisher PLSD post-hoc test (* P < .05; ** P < .0001). For the ABT 418 and A-81754 temperature data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,48)}$ = 11.52; P < .0001 for 20 min and $F_{(6,48)}$ = 6.48; P < .0001 for 60 min] and Fisher PLSD post-hoc test (*P < .0001).

Compound	Horizontal Activity Counts	Rectal Temperature at 20 min	Rectal Temperature at 60 min
μmol/kg		°C	
Saline	6131 ± 345	37.0 ± 0.1	37.1 ± 0.1
(−)-Nicotine			
1.9	4274 ± 741**	37.0 ± 0.1	37.0 ± 0.1
6.2	3655 ± 615***	36.1 ± 0.1**	36.7 ± 0.3*
19.0	836 ± 326****	34.5 ± 0.2**	36.7 ± 0.1*
(+)-Nicotine			
1.9	4554 ± 273*	37.0 ± 0.1	37.1 ± 0.1
6.2	3838 ± 542**	37.0 ± 0.1	37.1 ± 0.1
19.0	2608 ± 223****	37.0 ± 0.1	37.0 ± 0.1
Saline	6028 ± 606	37.2 ± 0.04	37.1 ± 0.04
ABT 418			
1.9	4475 ± 484	37.1 ± 0.05	37.1 ± 0.06
6.2	4518 ± 723	37.0 ± 0.03	37.1 ± 0.05
19.0	2643 ± 509**	36.1 ± 0.24*	36.8 ± 0.14
A-81754			
1.9	4843 ± 511	37.0 ± 0.05	37.1 ± 0.04
6.2	5306 ± 169	37.1 ± 0.05	37.0 ± 0.03
19.0	4362 ± 700*	36.4 ± 0.19*	36.3 ± 0.26*

potencies of ABT 418 and its enantiomer in producing reductions in body temperature and locomotor activity did not differ as much as did the relative potencies of the two nicotine enantiomers.

ABT 418 (0.62, 1.9 and 6.2 μmol/kg i.p.) did not significantly affect the rotorod performance of mice at 15, 30 or 60 min after dosing (fig. 4). after a higher dose of ABT 418 (11.6 μmol/kg i.p.), rotorod performance was significanty decreased at 15 min after dosing but returned to control values at 30 and 60 minutes after dosing. As expected, diazepam significantly decreased the rotorod performance of mice.

Antinocciceptive activity. ABT 418 (0.62, 1.9 and 6.2 μmol/kg i.p.had no effect on paw-lick latency (data not shown). A dose of 19.0 μmol/kg i.p., significantly elevated paw-lick latency at 30, but not at 15 or 60 min after dosing. This isolated antinociceptive effect was not considered a pharmacologically meaningful event. Morphine produced statistically significant increases in paw-lick latency at 15, 30 and 60 min after dosing.

Lethality. The approximate lethal dose for (−)-nicotine was 70 ± 4 μmol/kg i.p., which was approximately 2-fold lower than that seen with ABT 418 (137 ± 5 μmol/kg). ABT 418, in turn, was lethal at a lower dose than its enantiomer, A-81754 (353 ± 7 μmol/kg). Death occurred rapidly and was typically preceded by locomotor seizures.

Seizure and anticonvulsant activity. Seizures produced by ABT 418 and (−)-nicotine were similar in appearance. They typically occurred within a minute or two of injection and were preceded by a period of quiescence and labored breathing followed by a brief episode of uncontrolled, very rapid locomotor activity, "a running fit," and a period of tonic/clonic activity. The ED_{50} (95% confidence intervals shown in brackets) for seizures for ABT 418 was 61.6 μmol/kg i.p. [50.7–74.8] which was somewhat higher than that for (−)-nicotine (40.8 μmol/kg [33.9–49.2]), although the difference in potency of these two compounds to produce seizures was not as great as the difference in their lethal doses. Preadministration of the nAChR channel blocker, mecamylamine (15 μmol/kg i.p.) substantially increased the seizure thresholds for both ABT 418 and (−)-nicotine, suggesting that the ABT 418-induced seizures are nicotinically mediated (mecamylamine + ABT 418 > 110 μmol/kg i.p.; mecamylamine + (−)-nicotine = 69.4 [63.0- 76.4]).

ABT 418 (0.62, 1.9, 6.2 and 19.0 μmol/kg i.p.) had no effect on the latency to the onset of the three pentylenetetrazol-

otine. Interestingly, A-81754 appeared to have a longer-lasting effect on body temperature than either ABT 418 or (−)-nicotine, as it was the only compound tested that produced similar hypothermia at both the 20-min and 60-min time points. ABT 418 and A-81754 also decreased locomotor activity (table 3), although on this measure, ABT 418 was somewhat more potent than its enantiomer, A-81754. Neither compound was as potent nor as effective as (−)-nicotine in reducing locomotor activity, with ABT 418 producing a reduction in locomotor activity comparable to that produced by (+)-nicotine. Interestingly, the
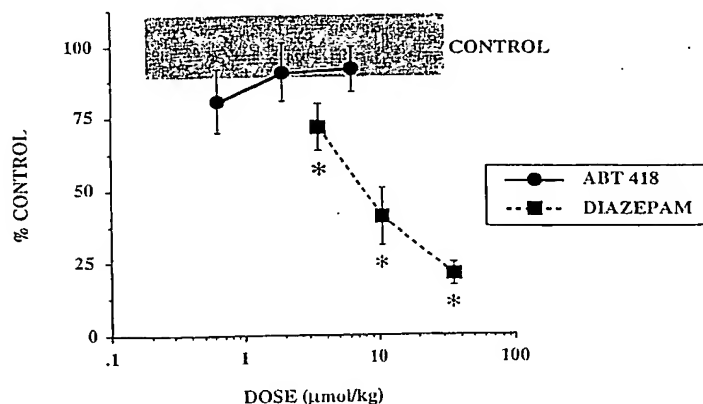

Fig. 4. ABT 418, in constrast to diazepam, does not impair rotorod performance in the anxiolytic dose range. Values are mean ± S.E.M.; n = 15; shaded area represents control mean ± S.E.M. *Different from control; P < .05.

induced seizure components: first-twitch, pseudoclonus and persistent convulsion (data not shown). A dose of 19.0 μmol/kg i.p., produced a small, statistically significant shortening of the latency to death after persistent convulsions. As expected, diazepam produced statistically significant delays in the onset of all three seizure components and death.

Rat Pharmacokinetics

Fifteen min after i.v. administration of 1.0 μmol/kg of ABT 418, plasma and brain levels of parent ABT 418 were nearly equivalent (plasma = 43 ± 4 ng/ml; brain = 50 ± 5 ng/g; n = 3). after administration of equivalent doses, plasma levels of (−)-nicotine were 50 ± 14 ng/ml (n = 3) similar to that seen with ABT 418. However, brain levels of (−)-nicotine were 282 ± 42 ng/g (n = 3), more than 5-fold greater than those seen in plasma. (−)-Nicotine also had twice the plasma half-life (42 ± 3 min; n = 4) of ABT 418 (23 ± 5 min; n = 6). In contrast, the oral bioavailability of (−)-nicotine (16 ± 5%; n = 2) was less than ABT 418 (27 ± 3%; n = 3).

Cerebral Blood Flow in Anesthetized Rat

At doses ranging from 0.002 to 2.0 μmol/kg i.v., ABT 418 had no effect on resting cerebral blood flow (table 4). Further, no effects on mean arterial blood pressure were observed over this dose range (table 4).

Electrical microstimulation of the basal forebrain elicits frequency-graded (6.25–50 Hz) increases in cortical cerebral blood flow up to 173 ± 8% of resting control values (n = 7–9) (table 4). These increases occurred in the absence of any observable change in mean arterial pressure on the original blood pressure trace (data not shown).

ABT 418, administered i.v., produced a 40% enhancement of the basal forebrain-elicited cortical cerebral blood flow response without significantly affecting resting cerebral blood flow (table 4). A significant increase was found at both 25 Hz (180 ± 10% of resting control value; n = 7) and at 50 Hz (202 ± 5% of resting control values; n = 7) after administration of 0.002 μmol/kg of ABT 418. The enhancement was not observed at higher doses.

EEG in Conscious Rat

The doses chosen for ABT 418 and (−)-nicotine were based on doses of these agents that maximally improve septal lesion-induced deficits in water maze performance in rats (Decker et al., 1992, 1994). The effects of ABT 418 and (−)-nicotine on free-run EEG were assessed for 20 min after injection using a dose of 1.9 μmol/kg i.p. for each compound (table 5). ABT 418

did not produce any significant effects in any of the frequency bands analyzed. In contrast, (−)-nicotine (1.9 μmol/kg i.p.) significantly reduced total power in the 1 to 4 (t = 5.4;, P < .001), 4 to 8 (t = 5.9; P < .001) and 8 to 13 Hz (t = 4.7; P = .001) bands and increased total power in the high frequency 25 to 50 Hz band (t= −2.52; P = .036). Thus, at equivalent doses known to enhance cognitive performance, these two nAChR ligands demonstrate a different profile of effects on free-run EEG.

Emetic Liability in Dog

(−)-Nicotine given in a dose of 500 nmol/kg i.v. elicited at least one bout of emesis in three of three dogs. In contrast, ABT 418 given at an equivalent dose did not produce emesis, or any other observable signs of gastrointestinal distress, in three of three dogs.

Discussion

ABT 418, a potent and selective neuronal nAChR ligand (Arneric' et al., 1994), shows stereoselective cognition-enhancing activity and nonsedating, anxiolytic-like activity in preclinical tests. This compound also enhances basal forebrain function as measured by increased basal forebrain-elicited cortical cerebral blood flow without causing generalized activation of the cortical EEG. ABT 418 has an improved safety profile relative to (−)-nicotine. A summary of the in vivo findings for ABT 418 is shown in table 6. These data suggest that ABT 418 does not elicit the dose-limiting side effects typically observed with (−)-nicotine (Arneric and Williams, 1993). The favorable behavioral effects of ABT 418 may be the result of potent activation of selective subtypes of nAChR, thereby maintaining the cognitive-enhancing and anxiolytic-like properties of (−)-nicotine while displaying an improved side effect profile relative to (−)-nicotine. Thus, ABT 418 may represent the first of a new class of nAChR channel ligands that have the potential of becoming safe and effective CNS therapeutic agents.

The current results indicate that memory, as assessed by retention of inhibitory avoidance training in mice, is improved by ABT 418. Administration of ABT 418 to mice before inhibitory avoidance training improved performance on a retention test conducted 24 hr later. The dose-response curve for this effect had an "inverted-U" shape that is characteristic of many memory-enhancing agents (Flood et al., 1981; Gold, 1989). The 10-fold difference between the minimally effective dose for ABT 418 in the current experiment (0.062 μmol/kg) and that of a related experiment with (−)-nicotine (0.62 μmol/kg) using

TABLE 4

Effects of ABT 418 on resting cortical cerebral blood flow, basal forebrain-elicited increased in cortical cerebral blood flow and mean arterial pressure

Values are mean ± S.E.M.; n = 7 to 9. All data are expressed as a percentage of resting control values.

Parameter	Control	Dose of ABT 418 (μmol/kg i.v.)			
		0.002	0.02	0.2	2.0
Resting cerebral blood flow	100.0 ± 1.8	116.4 ± 6.8	100.0 ± 8.9	100.5 ± 8.8	103.8 ± 10.0
Basal forebrain-elicited cerebral blood flow at 12.5 Hz	116.6 ± 2.4	135.1 ± 7.3	123.3 ± 9.9	121.8 ± 11.3	122.3 ± 10.7
Basal forebrain-elicited cerebral blood flow at 25 Hz	144.4 ± 9.0	179.7 ± 9.8*	152.3 ± 8.9	150.6 ± 6.1	160.0 ± 11.9
Basal forebrain-elicited cerebral blood flow at 50 Hz	173.5 ± 7.7	202.3 ± 5.4*	170.0 ± 10.0	168.8 ± 11.0	182.2 ± 14.9
Mean arterial pressure	98.9 ± 5.7	101.9 ± 3.1	87.8 ± 6.6	91.7 ± 6.7	88.3 ± 6.5

* $P < .05$ using ANOVA.

TABLE 5

Effects of ABT 418 and (−)-nicotine on free run EEG in rats

Values are mean ± S.E.M.; n = 8 to 9.

	Dose	Power				
		Frequency Band (Hz)				
		1–4	4–8	8–13	13–25	25–50
	μmol/kg i.p.			*μV*		
Control		14.9 ± 1.4	18.7 ± 2.4	8.1 ± 1.2	7.6 ± 1.1	3.3 ± 0.6
ABT 418	1.9	14.1 ± 1.6	17.2 ± 2.6	7.5 ± 1.2	5.8 ± 0.8	3.6 ± 0.2
(−)-Nicotine	1.9	10.5 ± 1.8*	12.9 ± 2.6*	4.3 ± 0.7*	5.3 ± 0.6	4.7 ± 0.6*

* P < .05, paired Students *t* test from saline control.

TABLE 6

Overview of the *in vivo* pharmacological properties of ABT 418 and the (R)-enantiomer, A-81754, compared to (−)-nicotine

ED_{min} is defined as the minimum dose of the drug that elicited a statistically significant response. ND, not determined; NA, not applicable.

Assay Procedure	ABT 418 (S-form)	A-81754 (R-form)	(−)-Nicotine (S-form)
Mouse inhibitory avoidance (ED_{min}, μmole/kg i.p.)	0.062	>0.62	0.62[a]
Mouse elevated plus-maze (ED_{min}, μmol/kg i.p.)	0.19	>6.2	0.62[b]
Rat cerebral circulation enhancement of basal forebrain vasodilation (ED_{max}, μmol/kg i.v.)	0.002	ND	0.43[c]
Toxicity (μmol/kg i.p., mice)			
ALD	138 ± 5	353 ± 7	70 ± 4
Seizure (ED_{50}; 95% confidence intervals)	62 [51–75]	ND	41 [34–49]
Hypothermia	19	19	6.2
Therapeutic index (inhibitory avoidance *vs.* ALD)	2225	NA	113
Emetic liability in dog (dogs responding to 500 nmol/kg i.v.)	0/3	ND	3/3
Pharmacokinetics			
RAT $t_{½}$	23 min	24 min	42 min
Bioavailability (p.o.)	27%	ND	16%

[a] From Brioni and Americ, 1993.
[b] From Brioni et al., 1993.
[c] From Linville et al., 1993.

identical conditions (Brioni and Arneric', 1993) strongly suggests that ABT 418 is more potent in improving retention of inhibitory avoidance training than (−)-nicotine. Although effects on nonassociative performance factors cannot be completely ruled out when drugs are administered before training as in the current set of experiments, it is unlikely that the improved performance found with ABT 418 resulted from a drug-induced increase in pain sensitivity because ABT 418 did not alter pain thresholds in the hot-plate test and did not alter shock sensitivity in pilot experiments (data not shown).

The enhanced retention test performance seen with ABT 418 was not observed with A-81754, the (R)-enantiomer of ABT 418, at doses up to 10 times the minimally effective dose of ABT 418, indicating that these effects are stereoselective. Furthermore, this effect of ABT 418 appears to be mediated by actions at nAChRs because mecamylamine, a nAChR channel blocker, completely prevented the effect of ABT 418. Cognitive-enhancing properties of ABT 418 are implicated by the improved retention of inhibitory avoidance training observed in the current study. The inhibitory avoidance task can be regarded as an example of simple fear conditioning but it still clearly involves a strong memory component, and effects of phamacological manipulations on this task are frequently consistent with effects on more complex measures of cognitive function. This latter generalization appears to be the case with ABT 418 as well, as preliminary reports suggest that ABT 418 improves delayed matching to sample performance in monkeys (J. J. Buccafusco, personal communication) and enhances spatial discrimination water maze learning in septal-lesioned rats (Decker *et al.*, 1994). ABT 418-induced memory enhancement *via* stimulation of nAChR would be consistent with a body of literature suggesting that nAChR agonists can improve cognitive performance in experimental animals (Haroutunian *et al.*, 1985; Elrod *et al.*, 1988; Levin *et al.*, 1990; Hodges *et al.*, 1992; Decker *et al.*, 1992) and in humans (Wesnes and Warburton, 1978, 1984; Rusted and Eaton-Williams, 1991).

ABT 418 has anxiolytic-like activity in the elevated plus-maze after systemic injection of 0.19 and 0.62 μmol/kg, doses that did not affect the general level of activity on the maze. This anxiolytic-like effect of ABT 418 is also stereoselective in that the (R)-enantiomer of ABT 418, A-81754, did not have anxiolytic-like activity. ABT 418 was 3 times more potent than (−)-nicotine and 15 times more potent than diazepam when compared across related experiments (Brioni *et al.*, 1993). In the present study, no effects on motor coodination were observed with ABT 418 in the dose range where anxiolytic activity occurred, which is in contrast to the effects observed with diazepam. Thus, contrary to the accepted notion that reduction of anxiety is associated with significant amnesia and motor incoordination (as in the case of diazepam, barbiturates and alcohol), activation of some nAChRs can produce anxiolytic-like effects without either sedation or memory impairment in rodents.

In addition to producing memory improvement and anxiolytic-like activity, ABT 418 enhanced the basal forebrain-elicited cortical cerebral blood flow response. The effects of ABT 418 on cerebral blood flow in rats were observed at lower doses than the effects of ABT 418 on plus-maze activity and retention of inhibitory avoidance in mice. In addition to the difference in the species used to assess these effects, it should also be noted that the blood flow experiments were conducted in anesthetized animals and the drug was injected i.v., both of which could have affected the dose response relationship. When compared to recently reported studies examining the effect of (−)-nicotine cortical cerebral blood flow (Linville *et al.*, 1993), the results of the present study suggest that ABT 418 is approximately 200 times more potent than (−)-nicotine in this para-

digm. The difference in potency between ABT 418 and (−)-nicotine in this model does not appear to be due to differences in the access of these two compounds to the brain after systemic administration. Furthermore, unlike (−)-nicotine, ABT 418 did not alter resting cerebral blood flow even at 10 times the dose that significantly enhanced the basal forebrain-elicited cortical cerebral blood flow response. Thus, ABT 418 appears to demonstrate some additional selectivity in its cerebral blood flow-enhancing effects by increasing basal forebrain-stimulated cortical cerebral blood flow without having nonspecific vasodilatory properties. ABT 418 would potentially be selective in enhancing cerebral blood flow regulated by the cholinergic basal forebrain-cortical system which exhibits profound impairment in AD. The utility of compounds that activate nAChR channels to ameliorate cerebral blood flow impairments in specific disease conditions such as AD may warrant further consideration. It is assumed that the observed cerebral blood flow impairments in AD are symptomatic of this disorder and do not represent the causative or primary insult to the CNS. However, the role of the cholinergic basal forebrain in the regulation of cortical cerebral blood flow, and the degree of impairment of this system in AD, in particular the loss of cortical nAChR and of cortical cerebral blood flow, suggest that efforts to recover the loss in cortical cerebral blood flow by pharmacotherapeutic intervention may also contribute to cognitive benefits.

(−)-Nicotine is known to produce cortical arousal activity which is characterized by a low amplitude EEG (Yamamoto and Domino, 1965). This was confirmed and quantified by calculating power (in μV) of EEG signals using fast Fourier transform analysis in the present study. (−)-Nicotine produced cortical arousal at a dose previously shown to elicit cognitive enhancement (Decker et al., 1992). Specifically, (−)-nicotine (1.9 μmol/kg i.p.) significantly reduced power in the lower frequency bands (1–4, 4–8 and 8–13 Hz) and increased power in the higher freqency bands (25–50 Hz). In contrast, the same dose of ABT 418 did not affect EEG power values in any of the frequency bands examined. Thus, ABT 418 displays an EEG profile that can be differentiated from (−)-nicotine. This may be of potential clinical importance for an aged patient population that already exhibits significant sleep disturbances and abnormal EEG patterns. Anecdotal reports suggest that (−)-nicotine patches cause similar sleep disturbances which, with chronic use, may lead to a further exacerbation of the cognitive and affective decline. ABT 418 may not cause the additional disruption of sleep that would come from elevated levels of (−)-nicotine (Soldatos et al., 1980).

Although doses of ABT 418 that have memory-enhancing, anxiolytic-like and cerebral blood flow-enhancing activities are lower than those required to obtain comparable effects with (−)-nicotine, ABT 418 is less potent than (−)-nicotine in producing acute toxicity. ABT 418 is significantly less potent than (−)-nicotine in producing overt seizure activity and death, although the separation between the potencies of these two compounds is somewhat less for seizure liability than for lethality. ABT 418-induced seizures appear to involve nAChRs because both (−)-nicotine- and ABT 418-elicited seizures were blocked by mecamylamine. Although high doses of ABT 418 did produce overt seizures, subeffective doses of the compound did not have proconvulsant actions when combined with pentylenetetrazol.

ABT 418 was also significantly less potent than (−)-nicotine in producing hypothermia and reducing locomotor activity.

Interestingly, A-81754, the (R)- enantiomer of ABT 418, was more toxic in these acute safety studies than would have been predicted from the binding studies. This enantiomer has a K_i value about 14 times higher than that for ABT 418 (Arneric' et al., 1994) but was only slightly less potent than ABT 418 in reducing temperature and activity and had a lethal dose only 2 to 3 times higher than that for ABT 418. This descrepancy could be related to actions by the enantiomer at other receptors or loss of stereoselectivity with high dose effects. Alternatively, A-81754 could be metabolized into a more toxic metabolite.

As previously discussed, clinical data suggest that (−)-nicotine can improve cognitive function in both normal people and AD patients. Similarly, (−)-nicotine has anxiolytic-like effects in stressful situations (Gilbert, 1979; Gilbert et al., 1989). However, there are several key issues related to administering (−)-nicotine or other nicotine-like compounds to a predominantly non-smoking, aged patient population over the course of a chronic disorder such as AD. As Sunderland et al. (1988) have noted, in AD patients, alterations in mood and cardiovascular liabilities associated with high doses of (−)-nicotine would make this a difficult drug to use on a chronic basis. With respect to the cardiovascular system there is abundant evidence that (−)-nicotine activates the sympathetic nervous system both through central activation of sympathetic outflow and indirectly through the enhanced release of circulating catecholamines (Benowitz, 1992). These actions can result in elevations in blood pressure and elevated levels of lipids that would potentially predispose an individual to atherosclerosis, and an increased workload to the heart (Benowitz, 1992) and may also lead to acute myocardial infarction and sudden death. There are also gastrointestinal and addiction liabilities associated with (−)-nicotine. (−)-Nicotine patches given to non-smokers frequently cause nausea and complaints of gastrointestinal distress. Although addiction liability may be of concern to a non-AD patient population, AD patients who previously smoked appear to lose the drive to continue smoking (Barclay and Kheyfets, 1988). However, it is clear that for maximal efficacy to be achieved, side-effect liabilities will have to be minimal. Although the improved safety profile of ABT 418 in rodents is not necessarily predictive of effects that might be found in humans, it is notable that early phase I safety testing in healthy young volunteers indicates that ABT 418 appears to be very well tolerated (Sebree et al., 1993), and reduced side effect liabilities of ABT 418 would provide a significant advantage for this agent in the treatment of AD.

In summary, in rodents ABT 418 improves retention of inhibitory avoidance training, has anxiolytic-like effects as measured by the elevated plus-maze and enhances basal forebrain stimulation-induced cortical cerebral blood flow. ABT 418 is at least as potent as (−)-nicotine in producing all of these potentially beneficial effects but is significantly less potent than (−)-nicotine in producing several undesirable side effects in rodents. The unique pharmacological profile of this compound could represent a significant advantage for the safe and effective therapeutic approach for the amelioration of cognitive and emotional disturbances accompanying AD or other related CNS disorders.

References

ARAUJO, D. M., LAPCHAK, P. A., ROBITAILLE, Y., GAUTHIER, S. AND QUIRION, R.: Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. J. Neurochem. 50: 1914–1923, 1988.

ARNERIC, S. P., SULLIVAN, J. P., BRIGGS, C. A., DONNELLY-ROBERTS, D., ANDERSON, D. J., RASZKIEWICZ, J. L., HUGHES, M. L., CADMAN, E. D., ADAMS, P., GARVEY, D. S., WASICAK, J. T. AND WILLIAMS, M.: (S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl) Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: I. *In Vitro* Characterization. J. Pharmacol. Exp. Ther. 270: 310–318, 1994.

ARNERIC, S. P. AND WILLIAMS, M.: Nicotinic agonists in Alzheimer's disease: Does the molecular diversity of nicotine receptors offer the opportunity for developing CNS selective cholinergic channel activators? *In* Recent Advances in the Treatment of Neurodegenerative Disorders and Cognitive Function, Capri, Italy, 1993.

AUBERT, I., ARAUJO, D. M., CECYRE, D., ROBITAILLE, Y., GAUTHIER, S. AND QUIRION, R.: Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. J. Neurochem. 58: 529–541, 1992.

BARCLAY, L. AND KHEYFETS, S.: Tobacco use in Alzheimer's disease. Alzheimer's Disease and Related Disorders 2: 172–178, 1988.

BARTUS, R. T., DEAN, R. L., III, BEER, B. AND LIPPS, A. S.: The cholinergic hypothesis of geriatric memory dysfunction. Science (Washington, DC) 217: 408–417, 1982.

BARTUS, R. T., DEAN, R. L., PONTECORVO, M. J. AND FLICKER, C.: The cholinergic hypothesis: A historical overview, current perspective, and future directions. Ann. N. Y. Acad. Sci. 444: 332–358, 1985.

BENOWITZ, N. L.: Nicotine and coronary heart disease. Trends Cardiovasc. Med. 1: 315–321, 1992.

BRIONI, J. D., ARNERIC, S. P.: Nicotinic receptor agonists facilitate retention of avoidance training: Participation of dopaminergic mechanisms. Behav. Neural. Biol. 59: 57–62, 1993.

BRIONI, J. D., O'NEILL, A., KIM, D. J. B. AND DECKER, M. W.: Nicotinic receptor agonists exihibit anxiolytic-like effects on the elevated plus-maze. Eur. J. Pharmacol. 238: 1–8, 1993.

BUCCAFUSCO, J. J. AND JACKSON, W. J.: Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in the young and aged monkeys. Neurobiol. Aging 12: 233–238, 1991.

COYLE, J. T., PRICE, D. L. AND DELONG, M. R.: Alzheimer's disease: A disorder of cortical cholinergic innervation. Science (Washington, DC) 219: 1189–1190, 1983.

DASTUR, D. K.: Cerebral blood flow and metabolism in normal human aging, pathological aging and senile dementia. J. Cereb. Blood Flow Metab. 5: 1–9, 1985.

DECKER, M. W., BRIONI, J. D., BUCKLEY, M. J., CURZON, P., AND ARNERIC', S. P.: Preclinical cognitive effects of cholinergic channel ligands. Abstracts of the XIXth C. I. N. P. Congress, 1994, in press.

DECKER, M. W. AND MAJCHRZAK, M. J.: Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotinic cholinergic antagonist, on spatial memory in rats. Psychopharmacology 107: 530–539, 1992.

DECKER, M. W., MAJCHRZAK, M. J. AND ANDERSON, D. J.: Effects of nicotine on spatial memory deficits in rats with septal lesions. Brain Res. 572: 281–285, 1992.

DECKER, M. W., MAJCHRZAK, M. J., AND ARNERIC', S. P.: Effects of lobeline, a nicotinic receptor agonist, on learning and memory. Pharmacol. Biochem. Behav. 45: 571–576, 1993.

ELROD, K., BUCCAFUSCO, J. J. AND JACKSON, W. J.: Nicotine enhances delayed matching-to-sample performance in primates. Life Sci. 43: 277–287, 1988.

FLOOD, J. F., LANDRY, D. W. AND JARVIK, M. E.: Cholinergic receptor interactions and their effects on long-term memory processing. Brain Res. 215: 177–185, 1981.

GARVEY, D. S., WASICAK, J., DECKER, M. W., BRIONI, J. D., SULLIVAN, J. P., CARRERA, G. M., HOLLADAY, M. H., ARNERIC, S. P. AND WILLIAMS, M: Novel isoxazoles which interact with brain cholinergic channel receptors have intrinsic cognitive enhancing and anxiolytic activities. J. Med. Chem., 37: 1055–1059, 1994.

GEANEY, D. P., SOPER, N., SHEPSTONE, B. J. AND COWEN, P. J.: Effect of central cholinergic stimulation on regional cerebral blood flow in Alzheimer disease. Lancet 335: 1484–1487, 1990.

GILBERT, D. G.: Paradoxical tranquilizing and emotion-reducing effect of nicotine. Psychol. Bull. 86: 643–661, 1979.

GILBERT, D. G., ROBINSON, J. H., CHAMBERLIN, C. L., SPIELBERGER, C. D.: Effects of smoking nicotine on anxiety, heart rate and lateralization of EEG during a stressful movie. Psychophysiology 26: 311–319, 1989.

GITELMAN, D. R. AND PROHOVNIK, I.: Muscarinic and nicotinic contributions to cognitive function and cortical blood flow. Neurobiol. Aging 13: 313–318, 1992.

GOLD, P. E.: Neurobiological features common to memory modulation by many treatments. Animal Learning Behav. 17: 94–100, 1989.

HAMEL, E. AND ESTRADA, C.: Cholinergic innervation of pial and intracerebral blood vessels: Evidence, possible origins and sites of action. *In* Neurotransmission and Cerebrovascular Function, Vol. II, ed. by J. Seylaz and R. Sercombe, pp. 151–173, Elsevier Science Publishers, New York, 1989.

HAROUTUNIAN, V., BARNES, E. AND DAVIS, K. L.: Cholinergic modulation of memory in rats. Psychopharmacology 87: 266–271, 1985.

HEISS, W.-D., SZELIES, B., ADAMS, J., KESSLER, G., PAWLIK, G. AND HERHOLZ,

K.: PET scanning for the detection of Alzheimer's disease. *In* Early Markers in Parkinson's and Alzheimer's Diseases (New Vistas in Drug Research, Vol. 1), ed. by P. Sostert, P. Riederer, M. Strolin-Benedetti and R. Roncucci, pp. 181–196, Springer-Verlag, Austria, 1990.

HODGES, H., SINDEN, J., TURNER, J. J., NETTO, C. A., SOWINSKI, P. AND GRAY, J. A.: Nicotine as a tool to characterize the role of the basal forebrain cholinergic projection system in cognition. *In* The Biology of Nicotine: Current Research Issues, ed. by P. M. Lippiello, A. C. Collins, J. A. Gray and J. H. Robinson, pp. 157–182, New York, Raven Press, 1992.

JONES, B. J. AND ROBERTS, D. J.: The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod. J. Pharm. Pharmacol. 20: 302–304, 1967.

JONES, G. M. M., SAHAKIAN, B. J., LEVY, R., WARBURTON, D. M. AND GRAY, J. A.: Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology 108: 485–494, 1992.

LEE, T. J.-F.: Cholinergic mechanism in the large cat cerebral artery. Circ. Res. 50: 870–879, 1982.

LEVIN, E. D.: Nicotinic systems in cognitive function. Psychopharmacology 108: 417–431, 1992.

LEVIN, E. D., CASTONGUAY, M. AND ELLISON, G. D.: Effects of the nicotinic receptor blocker, mecamylamine, on radial-arm maze performance in rats. Behav. Neural Biol. 48: 206–212, 1987.

LEVIN, E. D., LEE, C., ROSE, J. E., REYES, A., ELLISON, G., JARAVIK. M. AND GRITZ, E.: Chronic nicotine and withdrawal effects on radial-arm performance in rats. Behav. Neurol. Biol. 53: 269–276, 1990.

LINVILLE, D. G. AND ARNERIC, S. P.: Cortical cerebral blood flow governed by the basal forebrain: age-related impairments. Neurobiol. Aging 12: 503–510, 1991.

LINVILLE, D. G., WILLIAMS, S., RASKIEWICZ, J. L. AND ARNERIC, S. P.: Nicotinic agonists modulate basal forebrain (BF) control of cortical cerebral blood flow in anesthetized rats. J. Pharmacol. Exp. Ther. 267: 440–448, 1993.

MEYER, E. M., DE FIEBRE, C. M., HUNTER, B. E., SIMKINS, C. E., FRAUWORTH. N. AND DE FIEBRE, N. E. C.: Effects of anabaseine-related analogs on rat brain nicotinic receptor binding and on avoidance behaviors. Drug Devel. Res. 31: 127–134, 1994.

NEWHOUSE, P. A., POTTER, A., CORWIN, J. AND LENOX, R.: Acute nicotinic blockade produces cognitive impairment in normal humans. Psychopharmacology 108: 480–484, 1992.

NEWHOUSE, P. A., SUNDERLAND, T., TARIOT, P. N., BLUMHARDT, C. L. WEINGARTNER, H., MELLOW, A. AND MURPHEY, D. L.: Intravenous nicotine in Alzheimer's disease: A pilot study. Psychopharmacology 95: 171–175, 1988.

OLIVERIO, A.: Effects of mecamylamine on avoidance conditioning and maze learning of mice. J. Pharmacol. Exp. Ther. 154: 350–356, 1966.

PELLOW, S., CHOPIN, P., FILE, S. E., BRILEY, M.: Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14: 149–167, 1985.

PERRY, E. K., TOMLINSON, B. E., BLESSED, G., BERGMANN, K. AND GIBSON, P. H.: Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2: 1457–1459, 1978.

PETIT, D., LORRAIN, D., GAUTHIER, S. AND MONTPLAISIR, J.: Regional spectral analysis of the REM sleep EEG in mild to moderate Alzheimer's disease. Neurobiol. Aging 14: 141–146, 1993.

PINARD, E.: Cholinergic innervation of cerebral blood vessels: Functional aspects. *In* Neurotransmission and Cerebrovascular Function, Vol. II, ed. by J. Seylaz and R. Sercombe, pp. 175–191, Elsevier Science Publishers, New York, 1989.

PROHOVNIK, L., MAYEUX, R., SACKEIM, H. A., SMITH, G., STERN, Y. AND ALDERSON, P. O.: Cerebral perfusion as a diagnostic marker of early Alzheimer's disease. Neurology 38: 931–937, 1988.

RADEK, R.: Effects of nicotine on cortical high voltage spindles in rats. Brain Res., 625: 23–28, 1993.

RIEKKINEN, P., JR., SIRVIÖ, J., AALTONEN, M. AND RIEKKINEN, P.: The effects of concurrent manipulations of nicotinic and muscarinic receptors on spatial and passive avoidance learning. Pharmacol. Biochem. Behav. 37: 405–410, 1990.

RISBERG, J., GUSTAFSON, L. AND BRUN, A.: High resolution regional cerebral blood flow measurements in Alzheimer's disease and other dementia disorders. *In* Alzheimer's Disease. Epidemiology, Neuropathology, Neurochemistry, and Clinics (Key Topics in Brain Research), ed. by L. Maurer, P. Riederer and H. Beckmann, pp. 509–516, Springer-Verlag, New York, 1990.

RUSTED, J. AND EATON-WILLIAMS, P.: Distinguishing between attentional and amnestic effects in information processing: The separate and combined effects of scopolamine and nicotine on verbal free recall. Psychopharmacology 104: 363–366, 1991.

SAHAKIAN, B., JONES, G., LEVY, R., GRAY, J. AND WARBURTON, D.: The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. Br. J. Psychiatry 154: 797–800, 1989.

SCHRODER, H., GIACOBINI, E., STRUBLE, R. G., ZILLES, K. AND MAELICKE, A.: Nicotinic cholinoceptive neurons of the frontal cortex are reduced in Alzheimer's disease. Neurobiol. Aging 12: 259–262, 1991.

SEBREE, T., GREBB, J., KITTLE, C., AND KASHKIN, K.: A phase I, single, rising-dose study of A-81418, a novel cholinergic channel activator (ChCA). American College of Neuropsychopharmacology, Hawaii, December, 1993.

SHIMOHAMA, S., TANIGUCHI, T., FUJIWARA, M. AND KAMEYAMA, M.: Changes

in nicotinic and muscarinic cholinergic receptors in Alzheimer-type dementia. J. Neurochem. **46**: 288–293, 1986.

SOLDATOS, C. R., KALES, J. D., SCHARF, M. B., BIXLER, E. O. AND KALES, A.: Cigarette smoking associated with sleep difficulty. Science (Washington, DC) **207**: 551–553, 1980.

SUNDERLAND, T., TARIOT, P. N. AND NEWHOUSE, P. A.: Differential responsivity of mood, behavior, and cognition to cholinergic agonists in elderly neuropsychiatric populations. Brain Res. Rev. **13**: 371–389, 1988.

TILSON, H. A., McLAMB, R. L., SHAW, S., RODGERS, B. C., PEDIADITAKIS, P. AND COOK, L.: Radial-arm maze deficits produced by colchicine administered into the area of the nucleus basalis are ameliorated by cholinergic agents. Brain Res. **438**: 83–94, 1988.

WESNES, K. AND WARBURTON, D. M.: The effects of cigarette smoking and nicotine tablets upon human attention. *In* Smoking Behavior: Physiological and Psychological Influences, ed. by R. E. Thornton, pp. 131–147, Churchill-Livingston, London, 1978.

WESNES, K. AND WARBURTON, D. M.: Effects of scopolamine and nicotine on human rapid information processing performance. Psychopharmacology **82**: 147–150, 1984.

WHITEHOUSE, P. J., MARTINO, A. M., ANTUONO, P. G., LOWENSTEIN, P. R., COYLE, J. T., PRICE, D. L. AND KELLAR, K. J.: Nicotinic acetylcholine binding sites in Alzheimer's disease. Brain Res. **371**: 146–151, 1986.

WHITEHOUSE, P. J., PRICE, D. L., CLARK, A. W., COYLE, J. T. AND DeLONG, M. R.: Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. **10**: 122–126, 1981.

WILLIAMS, M.: Tacrine - recommendation for approval. Curr. Opin. Invest. Drugs **2**: 541–544, 1993.

WOOLFE, G. AND MacDONALD, A. D.: The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther. **80**: 300–307, 1944.

YAMAMOTO, K. AND DOMINO, E. F.: .: Nicotine-induced EEG and behavioral arousal. Int. J. Neuropharmacol. **4**: 359–373, 1965.

Short communication

Effects of ABT-418, a novel cholinergic channel ligand, on place learning in septal-lesioned rats

Michael W. Decker *, Peter Curzon, Jorge D. Brioni, Stephen P. Arnerić

Neuroscience Research, Dept. 47W, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064, USA

Abstract

Septal lesions disrupt septohippocampal neurotransmission and impair spatial memory. (−)-Nicotine reduces the memory deficits but has substantial side effect liabilities. Previous studies have demonstrated that ABT-418 is a novel, selective ligand for neuronal nicotinic acetylcholine receptors. In the current study, ABT-418 (0.19 and 1.9 μmol/kg, i.p.) administered before training significantly attenuated lesion-induced deficits in a spatial discrimination version of the Morris water maze. As lesion-induced learning deficits might parallel the cognitive deficits characteristic of Alzheimer's disease, these results suggest that ABT-418 may be useful in the treatment of this condition.

Key words: Acetylcholine; Hippocampus; Medial septal area; Nicotinic acetylcholine receptor; Spatial memory

1. Introduction

Degeneration of basal forebrain cholinergic neurons innervating the cortex, amygdala, and hippocampus is the most prominent and best-substantiated disruption of neurotransmitter function found in Alzheimer's disease (Coyle et al., 1983). Elucidation of the role played by damage to the forebrain cholinergic system has been central in attempts to understand the cognitive deficits associated with this disease, and efforts to develop therapeutic strategies have often targeted this system. The primary focus in attempts to understand the cognitive effects of cholinergic function and to develop neurotransmitter 'replacement' therapies has been on muscarinic cholinergic function. This emphasis on muscarinic cholinergic function is based, in part, on the more than 10-fold greater abundance of muscarinic acetylcholine receptors relative to nicotinic acetylcholine receptors in the brain (Araujo et al., 1990) and the well-known amnestic effects of muscarinic antagonists (Drachman, 1977).

More recently, there has been increased interest in the potential role of nicotinic neurotransmission in the memory deficits associated with Alzheimer's disease, and there is growing evidence that nicotinic neurotransmission plays an important role in memory modulation (Levin, 1992). Mecamylamine, which blocks nicotinic acetylcholine receptor channels, produces deficits in many of the same memory tasks previously shown to be disrupted by muscarinic blockade. For example, mecamylamine disturbs radial maze performance (Levin, 1992) and disrupts acquisition of spatial information in the Morris water maze (Decker and Majchrzak, 1992) in rats. Furthermore, mecamylamine impairs cognitive function in humans (Newhouse et al., 1992). Conversely, nicotinic acetylcholine receptor activation appears to improve performance in animals with impaired cholinergic function. (−)-Nicotine reverses some of the cognitive deficits observed in rats with basal forebrain lesions (Decker et al., 1992a; Hodges et al., 1991), lesions which model in rats the cholinergic dysfunction characteristic of Alzheimer's disease. Moreover, the beneficial effect of (−)-nicotine on acquisition of a spatial discrimination in the Morris water maze in rats with septal lesions is observed with another nicotinic acetylcholine receptor agonist, (−)-lobeline (Decker et al., 1993). These findings suggest

* Corresponding author. Address: Dept. 47W, Bldg. AP-10, Abbott Laboratories, Abbott Park, IL 60064, USA. Tel. (708) 937-2422, fax (708) 937-9195.

that nicotinic acetylcholine receptor activation might offer an alternative treatment strategy in Alzheimer's disease, a view supported by recent findings that (–)-nicotine improves some aspects of cognitive function in Alzheimer's disease patients (Jones et al., 1992). The therapeutic potential of (–)-nicotine, however, is severely limited by a variety of side effects, including gastrointestinal and cardiovascular liabilities. Thus, the utility of nicotinic acetylcholine receptor activation as a therapeutic strategy is limited by the lack of safe and selective nicotinic acetylcholine receptor ligands.

ABT-418 is a potent and selective ligand at nicotinic acetylcholine receptors (Arnerić et al., 1994) that readily crosses the blood brain barrier and improves retention of inhibitory avoidance training in mice (Decker et al., 1994). The current study sought to determine if ABT-418 can improve the performance of behaviorally impaired animals. Thus, the effects of ABT-418 were studied in rats with lesions of the septal area, lesions which reduce cholinergic input to the hippocampus and impair performance on spatial memory tasks such as the spatial discrimination version of the Morris water maze (Decker et al., 1992b).

2. Materials and methods

2.1. Subjects

Male Wistar rats (250–350 g) were used in this experiment. The rats were obtained from Charles River Laboratories (Portage, MI) and maintained in a climate controlled facility with a 12/12 light:dark cycle (lights on at 06:00). Behavioral testing was conducted during the light portion of the day.

2.2. Surgery

Lesions of the septum were produced by delivering radiofrequency current. The rats were anesthetized with pentobarbital (55 mg/kg, i.p.), and a TC electrode (0.7 mm diameter, 1.5 mm tip exposure) was placed in the septum under stereotaxic control using the coordinates: 0.5 mm anterior to bregma, 0.0 mm lateral to the midline, and 6.5 mm ventral to the skull surface. Current sufficient to maintain a temperature of 63°C at the electrode tip was passed for 60 s using a model RFG-4A lesion maker (Radionics, Burlington, MA). Sham surgery was conducted by lowering an electrode to a point 1.0 mm above the target location for the lesions, but passing no current. At the end of the behavioral experiments, the rats were deeply anesthetized (pentobarbital, 100 mg/kg, i.p.) prior to being decapitated and the lesions were verified histologically in 60 μm thick sections stained with thionin.

2.3. Spatial discrimination water maze training

Beginning 7–10 days after surgery the rats were trained in a spatial discrimination version of the Morris water maze, as previously described (Decker et al., 1992a). The rats were first trained to find a visible escape platform in a round pool (180 cm in diameter and 60 cm high) filled to a depth of 37 cm with water (26 ± 1°C) rendered opaque by the addition of powdered milk. Following this habituation training, the rats were trained on the spatial discrimination water maze. For the spatial discrimination task, two visible platforms similar in appearance to the platform used in cue training were used. However, only one of the platforms provided a means of escape from the water: the other was made of expanded polystyrene and did not support the weight of the animal. The position of each platform relative to extramaze visual cues remained constant during training. Rats received six trials per day, with the start location being changed from trial to trial. Contacts with the incorrect platform were scored as errors.

Either of two doses of ABT-418 (0.19 or 1.9 μmol/kg, i.p.) or 0.9% saline was administered to septal-lesioned rats 15 min before training sessions conducted on each of 4 consecutive days. Sham-lesioned rats received saline injections before training.

2.4. Drugs

ABT-418 [(S)-3-Methyl-5(1-methyl-2-pyrrolidinyl)-isoxazole hydrochloride salt; formula weight = 202.7] was synthesized by Abbott Laboratories (Abbott Park, IL 60064-3500). Drugs were dissolved in 0.9% sterile saline and injected i.p. The doses were selected based on previous experience with (–)-nicotine (0.3 mg of nicotine base = 1.9 μmol) and expressed in μmol. Control injections consisted of saline (1.0 ml/kg).

2.5. Statistics

Error data from the water maze were analyzed using a two-way repeated measures ANOVA, with training session as the repeated measure and treatment group as the between group measure. Subsequent analysis of error performance was also conducted by separate one-way ANOVA for each of the four training days.

3. Results

3.1. Histological verification of lesions

The lesions in this experiment were fairly large, destroying most of the medial portion of the septal area and frequently involving portions of the lateral
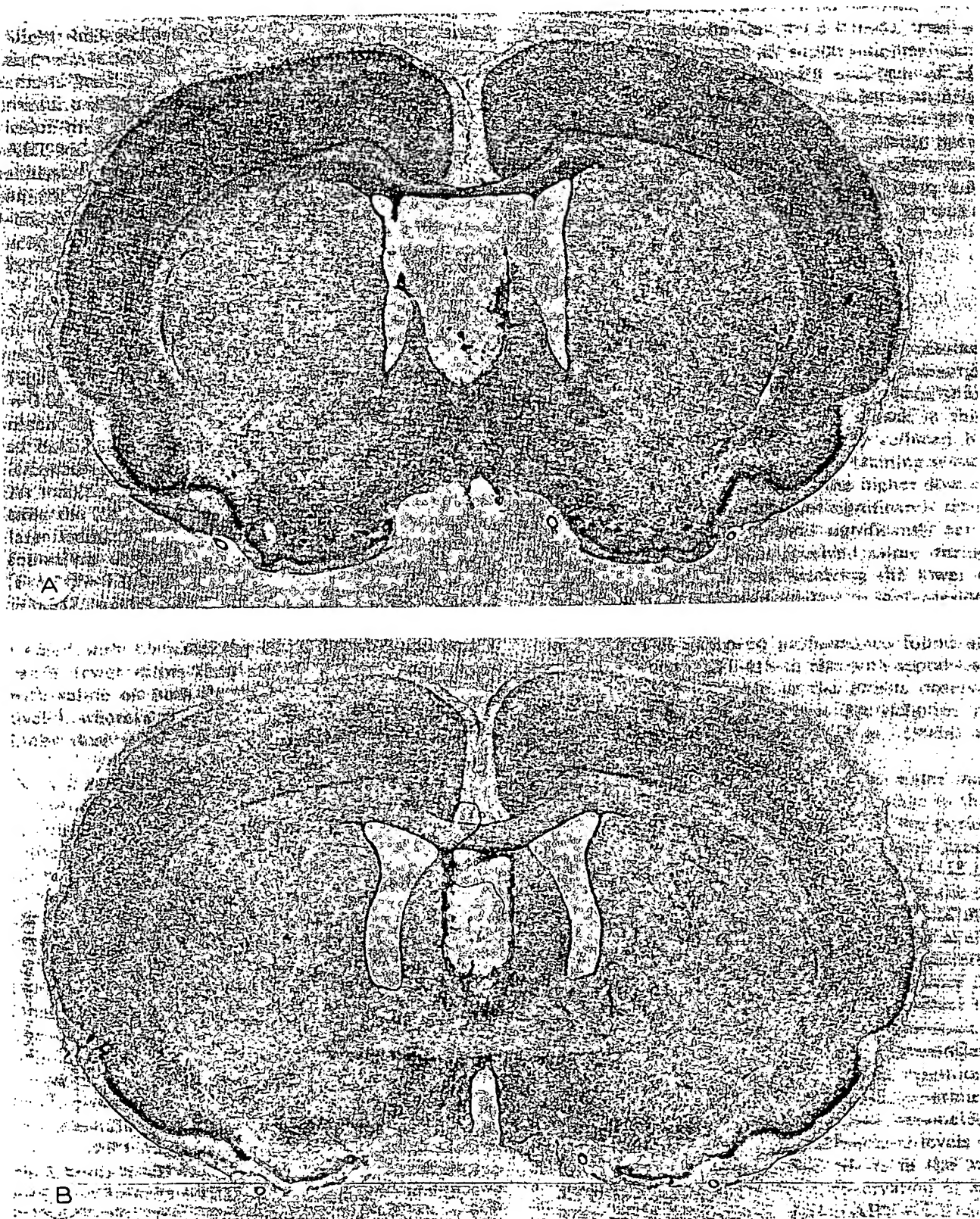
Fig. 1. Photomicrographs of a representative septal lesion. These Nissl-stained sections are from an animal who received the higher dose of ABT-418 (1.9 μmol/kg) during training.

septum and portions of the diagonal band of Broca. Slight damage to the corpus callosum was observed, and very little damage to the anterior commissure was noted. Some lesions extended caudally into the most rostral aspects of the fimbria-fornix. A representative lesion from a rat treated with the higher dose of ABT-418 is shown in Fig. 1. Although biochemical analysis was not conducted on the brains of these animals, previous studies from this laboratory demonstrate a 65–75% reduction of hippocampal choline acetyltransferase activity with lesions of this size (Decker et al., 1992a,b).

3.2. Spatial discrimination water maze

Analysis of the water maze training data shown in Fig. 2 reveals a main effect of treatment group [F(3,27) = 3.838, p = 0.021]. Overall, septal-lesioned rats that received saline during training were impaired relative to both sham rats given saline injections and septal lesioned rats that received 1.9 μmol/kg of ABT-418. As would be expected with an incrementally learned task, the lesion and drug effects were restricted to the later training sessions: Significant group effects were found on days 3 [F(3,27) = 4.957, p = 0.007] and 4 [F(3,27) = 5.107, p = 0.006] but not on days 1 [F(3,27) = 0.357, p = 0.784] and 2 [F(3,27) = 1.851, p = 0.162]. Post-hoc analysis revealed that septal-lesioned rats treated with 1.9 μmol/kg of ABT-418 made significantly fewer errors than septal-lesioned rats treated with saline on both days 3 and 4 (p < 0.01, respectively), whereas septal-lesioned rats treated with the lower dose of ABT-418 (0.19 μmol/kg) performed

Fig. 2. Effects of ABT-418 (0.19 and 1.9 μmol/kg) administered i.p. prior to each training session on learning a spatial discrimination water maze task in septal-lesioned rats. n = 7–8 per group. * Significant drug effect (different from lesion/saline treatment, p < 0.05); ** significant drug effect (different from lesion/saline treatment, p < 0.01).

significantly better than septal-lesioned rats treated with saline only on day 4 (p < 0.05). The improved performance observed in septal-lesioned rats treated with ABT-418 contrasts with the lack of effect we observed in a subsequent experiment with unoperated Wistar rats. Over the course of 4 days of training, unoperated rats treated with ABT-418 did not perform significantly differently from rats treated with saline (4 day mean ± s.e.m. = 2.79 ± 0.41 for saline, 2.79 ± 0.37 for 0.62 μmol/kg ABT-418, and 2.96 ± 0.25 for 1.9 μmol/kg ABT-418; n = 6 per group).

4. Discussion

As has been demonstrated previously (Decker et al., 1992b), septal lesions, which reduce cholinergic input to the hippocampus, impaired the acquisition of a spatial discrimination water maze task in this study. This impairment was significantly reduced by treatment with ABT-418 before each training session. Septal-lesioned rats that received the higher dose of ABT-418 did not at any point differ significantly from sham-lesioned rats and performed significantly better than septal-lesioned rats that received saline during training. Septal-lesioned rats receiving the lower dose of ABT-418 were improved relative to septal-lesioned rats receiving saline during training only on the last day of training. The improved performance found after administration of ABT-418 to rats with septal lesions on this task is comparable to the effects observed with similar doses of the nicotinic acetylcholine receptor agonists, (–)-nicotine (Decker et al., 1992a) and (–)-lobeline (Decker et al., 1993).

Although ABT-418 improved the water maze performance of rats with substantial damage to the septal hippocampal system, it did not alter the performance of intact animals tested in a subsequent experiment. The failure to observe an effect of ABT-418 in intact animals in the water maze contrasts with the previous finding that ABT-418 improves retention of inhibitory avoidance training in normal mice (Decker et al., 1994). In these previous studies, ABT-418 administered prior to training improved retention of inhibitory (passive) avoidance training in normal mice, an effect that was stereoselective and blocked by the nicotinic acetylcholine receptor channel antagonist, mecamylamine. It is important to note, however, that retention of inhibitory avoidance training in these experiments was adjusted by manipulating the shock parameters. Control performance was set at suboptimal levels in order to assess memory enhancing effects in the inhibitory avoidance experiments. Thus, observation of improved water maze performance during ABT-418 treatment in lesioned but not in unoperated rats could be related to the high level of performance typically observed in
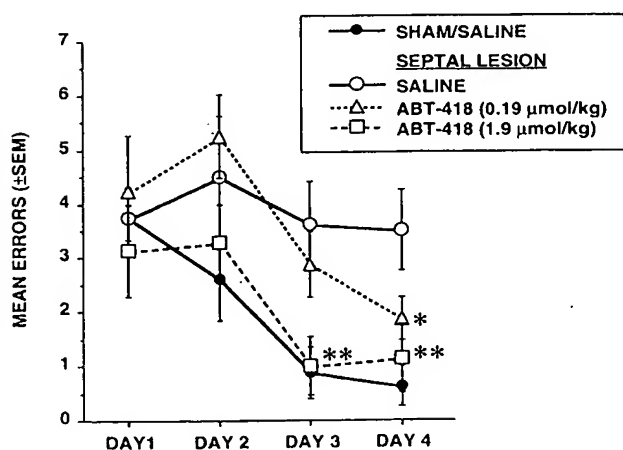
normal animals on this task. Thus, ABT-418 may be more effective in animals with performance deficits.

As with any compound administered before training, the effects of ABT-418 in the present experiment could be related to effects on nonassociative, performance variables such as motor function. However, performance in the current experiment was evaluated using an accuracy measure rather than latency measures often used to assess water maze performance. An accuracy measure was selected to minimize the influence of any effects of ABT-418 on motor performance.

Notably, ABT-418 appears to be at least as potent as (−)-nicotine in enhancing performance in rodent models of cognitive function, but has a significantly improved safety profile relative to (−)-nicotine (Decker et al., 1994). ABT-418 is less potent than (−)-nicotine in producing seizures, death, and reductions in locomotor activity and body temperature in mice (Decker et al., 1994). The relative potencies of ABT-418 and (−)-nicotine in in vitro functional assays differ according to the assay used, although the effects of both ABT-418 and (−)-nicotine in these assays are readily blocked by nicotinic acetylcholine receptor antagonists. For example, ABT-418 is about 10-fold less potent than (−)-nicotine in stimulating dopamine release from rat striatal slices but equipotent to (−)-nicotine in stimulating [^{86}Rb$^+$] efflux from mouse thalamus (Arnerić et al., 1994). These results suggest that ABT-418 and (−)-nicotine may have differential selectivity for nicotinic acetylcholine receptor subtypes, selectivity which may be the basis for the improved preclinical safety profile observed with ABT-418.

The site of action and the mechanisms by which ABT-418 induces improvement in the performance of septal-lesioned rats are not certain at present. Improved spatial memory task performance in septal-lesioned rats has also been observed with other compounds that potentially act by enhancing cholinergic function. The cholinesterase inhibitor, tetrahydroaminoacridine, a compound recently approved in the United States for the treatment of Alzheimer's disease, significantly attenuates the septal lesion effect on the hidden platform version of the Morris water maze (Riekkinen et al., 1990). This partial attenuation by tetrahydroaminoacridine differs from the more pronounced effect of ABT-418 in the current study. However, the differences between the behavioral procedures and potential differences in the size and placement of the lesions in these studies make it difficult to compare the efficacy of these two compounds in these preclinical impairment models. An improvement in the water maze performance of septal-lesioned rats has also been observed with linopirdine (DuP 966) under the same conditions used in the current study (Brioni et al., 1993). Linopirdine releases acetylcholine and several other neurotransmitters and is currently in clinical trials for the treatment of Alzheimer's disease. Amelioration of septal-lesion-induced deficits by compounds that activate nicotinic acetylcholine receptors [(−)-nicotine, (−)-lobeline, ABT-418], inhibit acetylcholine breakdown (tetrahydroaminoacridine), or release acetylcholine (linopirdine) suggests the possibility that potentiation of cholinergic neurotransmission might be responsible for the beneficial effects of these agents. However, it still remains to be determined whether the primary effect of these treatments is mediated through increased activation of acetylcholine receptors.

References

Araujo D.M., P.A. Lapchak, M.J. Meaney, B. Collier and R. Quirion, 1990, Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: Relationship to presynaptic cholinergic markers and binding sites, J. Neurosci. 10, 3069.

Arnerić S.P., J.P. Sullivan, C.A. Briggs, D. Donnelly-Roberts, D.J. Anderson, J.L. Raszkiewicz, M. Hughes, E.D. Cadman, P. Adams, D.S. Garvey, J. Wasicak and M. Williams, 1994, ABT 418: A novel cholinergic ligand with cognition enhancing and anxiolytic activity I. In vitro characterization. J. Pharmacol. Exp. Ther., in press.

Brioni J.D., P. Curzon, M.J. Buckley, S.P. Arneric and M.W. Decker, 1993, Linopirdine (DuP996) facilitates the retention of avoidance training and improves performance of septal-lesioned rats in the water maze, Pharmacol. Biochem. Behav. 44, 37.

Coyle J.T., D.L. Price and M.R. DeLong, 1983, Alzheimer's disease: a disorder of cortical cholinergic innervation, Science 219, 1184.

Decker M.W., J.D. Brioni, J.P. Sullivan, M.J. Buckley, R.J. Radek, J.L. Raszkiewicz, C.H. Kang, D.J.B. Kim, W.J. Giardina, M. Williams, S.P. Arnerić, 1994, ABT 418: A novel cholinergic ligand with cognition enhancing and anxiolytic activities: II. In vivo characterization, J. Pharmacol. Exp. Ther., in press.

Decker M.W. and M.J. Majchrzak, 1992, Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotinic cholinergic antagonist, on spatial memory in rats, Psychopharmacoloy 107, 530.

Decker M.W., M.J. Majchrzak and D.J. Anderson, 1992a, Effects of nicotine on spatial memory deficits in rats with septal lesions, Brain Res. 572, 281.

Decker M.W., M.J. Majchrzak and S.P. Arnerić, 1993, Effects of lobeline, a nicotinic receptor agonist, on learning and memory, Pharmacol. Biochem. Behav. 45, 571.

Decker M.W., R.J. Radek, M.J. Majchrzak and D.J. Anderson, 1992b, Differential effects of medial septal lesions on spatial-memory tasks, Psychobiology 20, 9.

Drachman D.A., 1977, Memory and cognitive function in man: does the cholinergic system have a specific role?, Neurology 27, 783.

Hodges H., Y. Allen, J. Sinden, P.L. Lantos and J.A. Gray, 1991, Effects of cholinergic-rich neural grafts on radial maze perfor-

mance of rats after excitotoxic lesions of the forebrain cholinergic projection system – II. Cholinergic drugs as probes to investigate lesion-induced deficits and transplant-induced functional recovery, Neuroscience 3, 609.

Jones G.M.M., B.J. Sahakian, R. Levy, D.M. Warburton and J.A. Gray, 1992, Effects of acute subcutaneous nicotine on attention, information processing short-term memory in Alzheimer's disease, Psychopharmacology 108, 485.

Levin E.D., 1992, Nicotinic systems and cognitive function, Psychopharmacology 108, 417.

Newhouse P.A., A. Potter, J. Corwin and R. Lenox, 1992, Acute nicotinic blockade produces cognitive impairment in normal humans, Psychopharmacology 108, 480.

Riekkinen P. Jr., J. Sirviö and P. Riekkinen, 1990, The effects of THA on medial septal lesion-induced memory defects, Pharmacol. Biochem. Behav. 36, 237.